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AN ADEQUATE LABORATORY SERVICE IN THE MODERN HOSPITAL*

BY WARD BURDICK,† M.D., DENVER, COLO.

AS the representative of the American Society of Clinical Pathologists, it becomes my privilege to convey the appreciation of your courtesy in extending the privilege of appearing before this convention for the purpose of expressing views on matters which so mutually concern us.

Upon receipt of the invitation to address this meeting on the subject of "An Adequate Laboratory Service in the Modern Hospital," a communication was directed to the Fellows of the American Society of Clinical Pathologists, enclosing a questionnaire covering this subject, and this paper is a digest of the answers received. It has been the endeavor to make it express the net result of the experiences of all of the Fellows of the Society which I represent.

The first question for discussion, it would seem, should be:

PERSONNEL

On this matter, there is a striking unanimity of opinion among the clinical pathologists. With reference to the head of the organization, O. J. West, Seattle, Washington, says: "All hospital laboratories should be in charge of a clinical pathologist on whom the responsibility for all work should rest." These words express the sentiment of all clinical pathologists from whom replies were received. This brings us to the pertinent question, "What is a clinical pathologist?" At the last Annual Convention of the American Society of Clinical Pathologists, the following definition was adopted:

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†Secretary of the American Society of Clinical Pathologists.

"A clinical pathologist is a physician who has specialized in clinical microscopy, chemistry, serology, bacteriology, pathologic histology, and gross morbid anatomy, for a period of two years under the direction of a clinical pathologist as herein defined."

Thus, at the outset, we find ourselves at variance with the American College of Surgeons on a very important point. Referring to the tenth Year Book of the College, issued during the present year, on page fifty-three, paragraph v, under the heading of "The Minimum Standard of Hospitals," reads as follows: "That clinical laboratory facilities be available for the study, diagnosis and treatment of patients, these facilities to include at least chemical, bacteriologic, serologic and histologic service in charge of trained technicians." John Hewat, Director of Laboratories, Department of Health, State of Maine, states: "If a hospital has a fully equipped laboratory capable of undertaking all laboratory procedures, the object of the laboratory is defeated, unless the responsible head is qualified, as defined by the American Society of Clinical Pathologists. The technical part of the work can, perhaps, be left to trained technicians, but a knowledge of the fundamentals of medicine is very essential in the interpretation of the results; therefore, the head of the laboratory should have a Medical Degree." "For," says James C. Todd, Professor of Clinical Pathology, University of Colorado, "it would seem reasonable to ask that all clinical laboratory work of a hospital be done under the direct control of a clinical pathologist, and that there be a definite department of clinical pathology recognized at staff meetings."

"In small hospitals," according to E. H. Buttles, Mary Fletcher Hospital, Burlington, Vermont, "perhaps under one hundred beds, he need not be a full-time man, but should have definite hospital hours." Harry Corper, National Jewish Hospital for Consumptives, Denver, insists that, "Every hospital of one hundred bed capacity should have a full-time resident clinical pathologist, with an appropriate staff of assistants." To these sentiments, every Fellow of the American Society of Clinical Pathologists subscribes, and we venture to say that were the American College of Surgeons to rewrite Paragraph v in the light of present day advancement in our field, the term "clinical pathologist" would supplant that of "technician."

That there is a place under the sun for the technician is the opinion of all clinical pathologists, for says Michael G. Wohl, Methodist Hospital, Omaha, "Lay technicians are valuable and absolutely necessary, yet when it comes to interpreting laboratory findings as clinical symptoms, a Degree in Medicine and several years' experience in hospital work are absolutely necessary"; but, J. H. Black, of Dallas, Texas, warns us that, "they should not be given autonomous positions." "Technicians," says A. H. Sanford, Mayo Clinic, Rochester, Minnesota, "are, in my experience highly trained laboratory workers, preferably women working under someone's direction." My personal opinion with reference to this matter is, it must be admitted, somewhat at variance with that of the majority of my colleagues, as it has long been the belief of the writer that the chief assistant in the hospital laboratory should be a potential clinical pathologist, a young man or woman who, having graduated in medicine and having served a proper internship in a general

hospital, elects to enter the field of clinical pathology as a life work. How else, it may be asked, may clinical pathology be perpetuated? The ever-widening circle of the application of laboratory methods to diagnosis, demands skilled physicians, especially trained to meet the demands of the modern hospital, but from whence are the clinical pathologists of the future to come? I do not know of a single young physician serving an apprenticeship in a laboratory. Instances may exist, and probably do, but they are hopelessly few. There are but two answers to this question, first, the encouragement of young physicians of scientific inclination to enter the laboratory as first assistants, and, second, to again quote James C. Todd, Professor of Clinical Pathology, University of Colorado, "See to it that there is a strong department of clinical pathology in every medical college."

Returning, then, to the subject, it may be said that the personnel of the hospital laboratory should include first, a clinical pathologist; second, to interpose a personal view, a young physician in course of training to become a clinical pathologist; and, third, as many technicians as the requirements of the institution may demand.

LOCATION, SIZE AND ARRANGEMENT, OF LABORATORY SPACE

That the laboratory should be on the uppermost floor, remote from the dust, noise and vibration of the street, with a northern exposure and located as near to the operating room as possible, is the consensus of opinion expressed by the clinical pathologists; Charles Sietz, Evansville, Indiana, says, "Its location should be such that it will be freely accessible to the members of the hospital staff, in order to invite and encourage consultation with the clinical pathologist. In the event of its not being convenient to place it near the operating room, a small laboratory for frozen sections of fresh surgical material should be provided adjoining the surgical department." There should be at least five hundred square feet for every one hundred beds, "and the arrangement," says John Kolmer, University of Pennsylvania, with whom most clinical pathologists agree, "should depend upon the service required; but for a one hundred and fifty bed hospital, I believe that the laboratory should be divided into the following departments with space set aside for each; (a) For the preparation of tissues and culture media, and general sterilization; (b) Clinical pathology and clinical bacteriology; (c) Serology, with special reference to the Wassermann test; (d) Biochemistry." To this allotment, many of our Fellows would add a private office and record room for the clinical pathologist in charge, and it is generally preferred that the above departments be provided with separate rooms. "In any event," says A. H. Sanford, Mayo Clinic, Rochester, Minnesota, "don't shunt the laboratory off into the basement."

"As to equipment," laconically replies one clinical pathologist, "the best is none too good"; but, W. W. Hall, Watertown, New York, advises, "the best of apparatus should be obtained; do not buy apparatus until there is a demand for it; discard apparatus as soon as defective, and always buy duplicate apparatus that is breakable. In the opinion of practically all of the clinical pathologists, however, the fundamentals of equipment are as

follows: Autoclave, Arnoll Sterilizer, Centrifuge, Incubator, Microscope, Refrigerator, Microtome, Basal Metabolometer, Colorimeter, Water-Still, Hemocytometers, and appropriate glass-ware, instruments, and chemicals for carrying on clinical microscopy, chemistry, serology, bacteriology, and pathologic histology, which, thinks A. V. St. George, Bellevue Hospital, New York City, "can usually be purchased for around $1,500.00. This would equip the average one hundred to one hundred fifty bed hospital laboratory. It is obvious, however, that the size of the institution will largely govern the amount of apparatus required." The writer would be inclined to make the latitude "around $1,500.00" rather liberal so that it might be stretched to $2,000.00 if need be, particularly in view of present prices.

SCOPE OF SERVICE

Now, in terms of golf, the ball has been knocked out into the rough, for here will be encountered several moot questions: first, what tests, if any, should be done as a matter of routine, taking into account both medical and surgical cases? "Routine blood and urine analysis," answers George L. Bond, Grand Rapids, Michigan, with whom ninety-six per cent of the clinical pathologists agree. "These tests reveal so many conditions that influence treatment, that the attending physician should have them in every case"; L. H. Cornwall, New York City Hospital, adds, "They are a necessary part of every record before diagnosis can be made with accuracy." Foy C. Payne, Dayton, Ohio, who voices the opinion of 47 per cent of his colleagues, states, "A Wassermann should be done routinely on all chronic cases." We are also reminded by several not to overlook a routine throat culture and vaginal smear in children, as well as the determination of coagulation and bleeding time on all tonsil and adenoid cases. Other tests, such as basal metabolism, blood chemistry, kidney function, blood typing, etc., were occasionally mentioned in the replies; but, A. B. Giordano, South Bend Medical Laboratory, South Bend, Indiana, says, "These three tests, namely, complete blood count, urine analysis and Wassermann reaction, will in a great many cases reveal evidence of disease process, of which neither the patient nor physician is aware." My own views as to hospital laboratory routine coincide with those expressed by John R. Porter, Rockford, Illinois, who says, "Urines and blood counts on all, sputum on all chest cases, blood culture on all temperatures of one hundred and two degrees Fahrenheit; throat cultures on all suspects and undiagnosed cases; stools on all diarrheas, and smears and cultures on all discharges; for the average clinician does not know exactly what laboratory examinations his patients should have, so he does not order them, and the above will many times clear up the case before the tests suggest themselves to him." "Furthermore," as Father Mouliner is wont to remind us, "the patient in the hospital is the object around which everything should revolve." The clinical pathologist should be one of the patient's closest relatives during his stay in the institution, and should not be hampered by formalities during the application of his endeavors to elucidate the cause of disease. His advances should be welcomed by attending physicians as an integral part of hospital service to which his patient is entitled.

With reference to surgical cases, it should be an axiom that no cutting operation, however small, should be undertaken—with the ever-present liability of infection—without first ascertaining the status of the blood as a matter of record; and, says Dr. Edward Mugrage, Assistant Professor of Clinical Pathology, University of Colorado, "The urine should always be checked in order to observe any renal disturbances which might otherwise be overlooked, and which would influence the choice of the anesthetic." It is the consensus of opinion that the histologic structure of all tissues removed at operation should be made a matter of record, not only as a check on clinical diagnosis, but as evidence which may be of inestimable value to the patient or his relatives at some future period, to say nothing of the statistical worth of such archives.

Another question: Should the activities of the hospital laboratory be limited to the institution alone, or may its scope of service reach out beyond the confines of the hospital? On this point, the clinical pathologists seem to be equally divided. "Most emphatically, yes!" says B. W. Rhamey, Fort Wayne, Indiana, representing one extreme, "They should confine their activities to the institution alone. Hospitals have no business going into competition with men making a specialty and life work of this branch of medicine. That would be another phase in the advance toward State Medicine"; but Ralph G. Stillman, Department of Pathology, New York Hospital, representing the other extreme, states, "I see no reason why the hospital laboratory should confine its activities to the institution, providing its personnel is large enough to handle the necessary work. The offer of diagnostic aid to practicing physicians in the neighborhood of the hospital, and on the hospital staff, broadens the service of the institution to the community, and establishes for the institution a permanently interested clientele that is of value both to the institution and to the community. No hospital that fails to act as a center for the dissemination of information concerning the prevention, diagnosis and treatment of disease is fulfilling its entire duty." I prefer the middle ground with M. W. Lyon, Jr., of South Bend, Indiana, who says that "if the clinical pathologist in the hospital is the only one in the community, he may reach outside; but, if there are private practicing clinical pathologists in the community, the hospital laboratory should keep to itself." For, after all, the development of clinical pathology as a specialty in medicine should not be impeded.

FINANCING

We are now face to face with another problem reflecting upon the scope of service of the hospital laboratory, namely, that of financing this department of the institution. This brings us deeper into the thicket of uncertainty, and constitutes the "fly in the ointment" of cooperation in this phase of hospital standardization.

Adequate laboratory service will not be realized until the true status of the clinical pathologist is recognized by all classes of physicians. From the most remote time up to about twenty years ago, the contact of the medical profession with the laity was chiefly through one man—the family doctor.

About this time, there appeared a third party, the clinical pathologist, whose function it was to develop methods for the practical application of scientific facts which were rapidly being brought to light by the medical research laboratories of the world. The newcomer was acclaimed by physicians and surgeons alike, who paid their compliments with a shower of material to examine gratis. But it was soon evident that a definite department of medicine was developing which required to be maintained and the present confusion proceeds from the reluctance on the part of the firmly grounded practitioner to so modify his demands upon the public that the clinical pathologist might derive his share of financial support. A comparison of the skill of the clinical pathologist involved in condemning a breast or a limb, with that of the surgeon who removes it, would be as likely to balance in favor of the former as the latter; but a considerable difference in compensation still obtains. That the public pays enough for medical service is obvious, and it is hoped that the handwriting on the wall will not escape the attention of those concerned, for the person who is not indifferent to the signs of the times may with safety predict that State Medicine will be the inevitable outcome if the cost of medical, and particularly surgical, service continues to increase.

As to methods in vogue at the present time for financing clinical pathology in the hospitals, an analysis of the questionnaires reveals a complete lack of uniformity, and one is at a loss to make any recommendations. For the present, however, we must proceed upon the thesis that the clinical pathologist is a consultant in medicine and should be compensated accordingly the same as the surgeon or internist. Regarding the so-called "flat fee," a majority of the clinical pathologists agree with Dr. Philip Hillkowitz of Denver, who says, "that a flat fee is permissible only for routine urine and blood examinations; that the hospital laboratory should be financed by charging each patient for work performed; while, for the more complicated tests, the usual fees in vogue should be charged and after proper deduction is made from the total monthly laboratory income for expense of laboratory supplies, salary of technicians, interest on investment, expense of rooms, light, heat, etc., the balance should be regarded as the compensation of the clinical pathologist."

In closing, may I suggest the appointment of a committee by the American College of Surgeons to cooperate with a similar committee from the American Society of Clinical Pathologists, to formulate a plan of financing the department of clinical pathology in the hospital?

To summarize, then, the personnel of the hospital laboratory should consist of a clinical pathologist in charge, a young physician in training for clinical pathology, and as many technicians as the work of the institution may require. It should be located adjacent to the operating room, preferably on the top floor, with a northern exposure, and subdivided into five rooms, including a private office for the clinical pathologist. The equipment should be adequate for the carrying on of all the necessary tests in clinical microscopy, pathologic histology, bacteriology, serology, and chemistry. As a matter of routine, the laboratory department should determine the condition of the

blood and urine of all patients entering the hospital, and write into the records of the institution the results of histologic examination of all tissues removed at operation. A Wassermann test should also be done as a matter of routine, at least upon chronic cases. The clinical pathologist should be freely employed as a consultant by the staff, and it should be considered his privilege and duty to perform any tests which, in his judgment might throw light on the condition of any patient. The activities of the hospital laboratory should be confined to the institution alone, unless it happens to be the only available center of clinical pathology in the community. The evolution of a satisfactory method of financing clinical pathology in the modern hospital is a matter for future determination and especially in the province of the committee suggested.

THE INFLUENCE OF THE NATURAL ANTISHEEP HEMOLYSINS OF HUMAN SERA UPON THE PRODUCTION OF ANOMALOUS REACTIONS IN THE FIRST TUBE OF KOLMER'S QUANTITATIVE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By Robert A. Kilduffe, A.B., A.M., M.D., Los Angeles, Calif.

IN his original communication detailing the technic of a new quantitative complement-fixation test for syphilis in which the quantitative factor is primarily dependent upon the testing of five graduated amounts of serum—0.1, 0.05, 0.025, 0.005, and 0.0025 c.c.—Kolmer[1] called attention to the fact that, occasionally, a positive serum will show less fixation in the first tube of the series carrying 0.1 c.c. of serum than in the second and subsequent tubes carrying 0.05 c.c. or less, thus producing such a reaction as, for example, 2 4 4 4 0.

This observation has been corroborated by all who have worked with the method and, in my own experience, occasional sera have even exhibited complete hemolysis in the first tube of the series when hemolysis has been inhibited to a greater or less degree in the subsequent tubes, thus producing such a reaction as 0 3 3 0 0 or 0 4 3 0 0.

The natural assumption is to ascribe this type of reaction to the presence of natural antisheep hemolysins in the serum tested in amounts sufficient in 0.1 c.c. to cause marked or even complete hemolysis of 0.5 c.c. of 2 per cent sheep-cell suspension, and to assume that the dilution of the serum in the subsequent tubes of the series so reduces the hemolysin content that its effect is masked or negligible.

The practical importance of such reactions lies in the fact that their occurrence furnishes a potential source of error in methods using only a single serum dose of 0.1 c.c.

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The assumption that these reactions are entirely due to the presence of natural antisheep hemolysins is logical and, apparently, reinforced by the fact that such reactions have not as yet been encountered with spinal fluids in which natural hemolysins are not found. It is opposed, however, by the fact that in the same communication Kolmer notes their occurrence when using an antihuman hemolytic system and also with hemolysin-free sera, as a result of which he believes them to be due, possibly, to the presence of other and as yet unknown serum constituents which may, in some way yet to be elucidated, interfere with the interaction of complement, antigen, and reagin.

A similar view is held by Detweiler[2] who calls attention to "some peculiar substance present in some sera which causes hemolysis in exactly the same fashion (as hemolysin) but which is not removed on exposure to sheep cells."

The same observer cites Zinnser[3] as also having noted the same phenomenon.

The occurrence of these anomalous reactions as brought out by Kolmer's quantitative method, while relatively infrequent, has a very distinct, important, and practical bearing on complement-fixation technic in general, for, if the reaction is in any way related to the hemolysin content of the serum tested, it becomes an important source of possible error in methods using only a single serum dose of 0.1 c.c.; and, even if it is not related to the presence of natural hemolysins, it still remains as a potential source of false negative reactions.

In the quantitative method described by Kolmer these anomalous reactions are of little practical importance as affecting the reading of the test, for the degree of positiveness is determined, primarily, not by the degree of fixation obtained in any one serum dose, but by the smallest quantity of serum which gives fixation of any degree. Thus, a 0 4 4 4 0 and 2 4 4 3 0 reaction would both be classed as very strongly positive because both show fixation in the fourth tube or in 0.005 c.c. of serum.

Because, however, such a reaction as 0 4 4 4 0 is a possible source of confusion to those accustomed to readings obtained with a single serum dose, and because of the inherent interest of the problem, it seemed of interest to study the problem and the present communication is the result of such a study.

In classing these reactions as a possible source of confusion it is, of course, plain that this is not as apt to be true of the serologist as of the clinician to whom the report is sent; but as long as the clinician is to assume, without exception, the sole prerogative of the interpretation of the complement-fixation reaction, and as long as too frequently, unfortunately, decisions relative to diagnosis and prognosis are to be based upon the results of isolated serologic examinations, it is necessary insofar as is possible, for the laboratory to bend every effort toward clarifying the methods of making and reading the results of the test.

The plan of this investigation was influenced by the following premises:

If the anomalous reactions in question are due entirely, or even in large measure, to the influence of natural hemolysins, then the incidence of both

should be comparable. Strict parallelism need not be looked for necessarily because of the influence of varying amounts of syphilitic reagin in the sera with resultant more or less complete and relatively stable fixation of complement for, while it has been shown that, within certain limits, a deficiency of complement may be compensated for by an excess of hemolysin and the reaction of a weakly positive serum thereby masked or even lost entirely, the influence of an excess of hemolysin upon the reaction obtained with a strongly positive serum containing large amounts of reagin is, to all intents and purposes, negligible, *especially when the serum is tested in amounts of less than 0.1 c.c.*

The plan adopted, then, was:

1. To determine the incidence of natural antisheep hemolysins in all the sera examined.

2. To determine the incidence of all sera giving anomalous reactions in the first tube.

3. To compare the incidence of 1 and 2.

4. To determine the incidence in the sera giving anomalous reactions of antisheep hemolysin in amounts sufficient in 0.1 c.c. to completely or even partially hemolyze 0.5 c.c. of 2 per cent sheep-cell suspension; to absorb the hemolysins from such sera, and retest them and thus determine the effect of absorption of the natural antisheep hemolysins on the reaction in the first tube of the quantitative test.

I. The Incidence of Natural Antisheep Hemolysins in Human Sera.

To determine this, to 0.1 c.c. of inactivated serum (15 minutes at 56° C.), were added 1 c.c. of 1:30 complement carrying two "full" units as determined by Kolmer's method of complement titration, and 0.5 c.c. of 2 per cent sheep-cell suspension, the tubes then being incubated for one hour in the water-bath at 38° C. Readings were then made for the presence and relative amount of natural antisheep amboceptor.

In all, a total of 2,180 sera were thus examined with the following results:

Sera containing sufficient hemolysin in 0.1 c.c. to cause complete hemolysis: 1,292 or approximately 50 per cent.

Sera containing sufficient hemolysin to cause marked but not complete hemolysis: 70 or approximately 3 per cent.

Sera containing only sufficient hemolysin to give a trace of hemolysis: 26 or approximately 1 per cent.

Sera without natural hemolysin: 729 or approximately 35 per cent.

The total incidence of hemolysin-containing sera was, therefore, 1,380 or approximately 60 per cent.

II. The Incidence of Anomalous Reactions in the First Tube of the Quantitative Test.

Of the 2,180 sera subjected to the quantitative complement-fixation test according to the method of Kolmer, anomalous reactions occurred in the first tube in 37 sera or approximately 1 per cent.

They may be divided into two groups: one group of eleven sera gave reactions characterized by complete hemolysis in the first tube with varying

degrees of fixation in the subsequent tubes of the series; and a second group, comprising twenty-six sera in which there was fixation in the first tube but always of lesser degree than that obtained in the second tube carrying 0.05 c.c. of serum.

It is highly important to note that, in the group of eleven sera giving complete hemolysis in the first tube, there were two sera which did not contain any natural hemolysin.

III. A comparison of the incidence of the natural antisheep hemolysin and of the incidence of anomalous reactions at once indicates that the presence of the former, even in large amount, cannot be the sole factor concerned in the production of the latter; for, while hemolysins were present in large amount in 1,292 sera, anomalous reactions occurred in only 37 sera.

Moreover, of the eleven sera giving complete hemolysis in the first tube, there were two which did not contain natural antisheep amboceptor and in which the reaction, therefore, cannot be ascribed to its presence and must be ascribed to unknown serum constituents other than hemolysin.

The results of the investigation thus far, therefore, suffice to clearly demonstrate the fact that anomalous reactions of the character in question may and do occur in the absence of natural antisheep hemolysin, and that the presence of natural hemolysin is not necessarily the cause of such reactions.

The relation of the natural amboceptor to the production of such reactions will be touched upon in the subsequent phases of the investigation detailed below.

IV. The incidence, in sera giving anomalous reactions, of antisheep hemolysins in amounts sufficient in 0.1 c.c. to completely hemolyze 0.5 c.c. of 2 per cent sheep-cell suspension, and the effect upon the anomalous reaction of the absorption of the natural amboceptor from the sera.

Of the 37 sera in question 34 contained sufficient hemolysin to completely hemolyze the test dose of cells. These were treated with sheep cells to absorb the amboceptor and retested for the degree of complement fixation after absorption.

At the outset of this portion of the study some little difficulty was encountered in finding a method satisfactory for the complete absorption of hemolysins without the coincident development of thermostabile anticomplementary properties in the serum after absorption.

A method was desired which would be rapid, simple, and efficacious and that of Kahn[4] was finally chosen. This method consists simply of the addition of washed and packed sheep cells to the serum in the proportion of one drop of cells to one centimeter of serum and the subsequent removal of the serum by centrifugation after standing for ten minutes at room temperature.

The cells were added to the serum from a 1 c.c. pipette graduated in $\frac{1}{100}$ c.c., both the serum and the cell quantities being approximated and not accurately measured.

Kahn and Lyon[5] have shown that this procedure is capable of absorbing

1,800 units of amboceptor from 1 c.c. of serum after ten minutes at room temperature, so that the method is efficacious as well as rapid and simple.

After absorption the sera were removed by centrifugation and stored in the ice chest overnight. The following morning the sera were again inactivated for 15 minutes at 56° C. and again tested by the quantitative method.

At the same time that the second test was made, to 0.1 c.c. of serum were added 1 c.c. of 1:30 complement carrying two "full" units, and 0.05 c.c. of 2 per cent sheep-cell suspension, the tubes then being incubated for one hour in the water-bath at 38° C., when a reading was made to determine if the amboceptor had been completely absorbed.

Absorption was found to have been complete in every instance and in no instance did the development of thermostabile anticomplementary substances occur.

The results of the complement-fixation tests before and after absorption are shown in the tables.

Over and above the question at issue, a study of the tables brings out several points of interest.

In Table I, while the sera in which natural hemolysins were absent demonstrate conclusively the fact that negative reactions may occur in the first tube through the action of unknown serum constituents, the remainder of the series evidences, by the results of the test after absorption, that the presence of natural antisheep hemolysins may be a factor of importance in the causation of this type of anomalous reaction, as fixation in varying degree was obtained in the first tube after absorption in every instance.

Table II brings out two facts of interest and importance:

a) While the strength of the reaction was definitely increased in the first tube as a result of absorption of hemolysin in 14 sera, in 12 sera no increase in the strength of the reaction occurred after absorption, so that, in approximately 50 per cent of these sera the anomalous reactions occurred independently of the presence of natural hemolysin, and these reactions, had the sera simply been tested for hemolysin content without absorption and retesting, would have been without hesitation attributed entirely to the hemolysin content.

This, however, was not the case and the mechanism of their occurrence remains to be explained.

b) In a perceptible number of the retested sera the strength of the reaction was slightly decreased as a result of absorption and reinactivation; for example, where fixation was obtained in the fourth tube before absorption, on retesting fixation did not extend beyond the third tube.

The explanation of this, in all probability, lies in the possible destruction of small amounts of reagin during the second inactivation period. In view of the fact, however, that the total period of exposure to 56° C. was not longer than that usually made use of heretofore, this explanation may not be the true one and other factors may be at work. It is possible, also, that the effect of exposure to heat may be more clearly brought out in the higher dilutions of the serum.

TABLE I

EFFECT OF ABSORPTION OF HEMOLYSIN ON SERA GIVING COMPLETE HEMOLYSIS IN THE FIRST TUBE

NO.	BEFORE ABSORPTION	AFTER ABSORPTION
1.	0 1 1 0 0	2 1 0 0 0
2.	0 1 4 4 2	4 4 4 0 0
3.	0 4 4 4 0	4 4 4 0 0
4.	0 4 4 4 0	2 2 2 0 0
5.	0 4 4 4 0	4 4 4 4 1
6.	0 4 4 0 0*	0 4 4 0 0
7.	0 2 4 1 0	4 4 4 0 0
8.	0 2 2 2 0	4 4 4 0 0
9.	0 1 1 1 0	4 4 4 4 0
10.	0 4 4 0 0	4 4 4 0 0
11.	0 4 4 0 0*	0 4 4 0 0

*Serum does not contain natural antisheep hemolysin.

TABLE II

EFFECT OF ABSORPTION OF HEMOLYSIN ON SERA GIVING LESS FIXATION IN TUBE 1 THAN IN TUBE 2

NO.	BEFORE ABSORPTION	AFTER ABSORPTION
1.	2 4 4 0 0	2 4 4 0 0
2.	1 3 3 1 1	4 4 0 0 0
3.	1 4 4 0 0	1 4 4 0 0
4.	3 4 4 0 0	4 4 4 0 0
5.	3 4 4 0 0	3 4 4 0 0
6.	2 1 1 1 0	4 4 4 0 0
7.	1 4 4 0 0	1 4 4 0 0
8.	1 4 4 0 0*	1 4 4 0 0
9.	3 4 3 2 0	4 4 4 0 0
10.	1 4 4 0 0	4 0 0 0 0
11.	1 4 4 0 0	4 4 4 0 0
12.	2 4 0 0 0	4 2 0 0 0
13.	1 4 0 0 0	4 1 0 0 0
14.	1 4 0 0 0	1 4 0 0 0
15.	2 3 2 2 2	2 3 3 3 0
16.	1 2 2 2 0	4 4 4 1 0
17.	1 2 0 0 0	4 0 0 0 0
18.	1 4 4 0 0	4 4 4 0 0
19.	3 4 0 0 0	4 4 4 0 0
20.	1 2 0 0 0	1 2 0 0 0
21.	3 4 0 0 0	3 4 0 0 0
22.	3 4 0 0 0	3 4 0 0 0
23.	1 2 1 0 0	4 4 4 0 0
24.	1 2 0 0 0	1 2 0 0 0
25.	1 2 0 0 0	4 4 0 0 0
26.	1 2 0 0 0	4 4 0 0 0

*Serum does not contain natural antisheep hemolysin.

This finding is purely of interest only in connection with the interpretation of the reaction as there will be but little difference in the clinical significance of a four-tube or a five-tube fixation—both will be very strongly positive and the patient in dire need of systematic treatment.

CONCLUSIONS

1. In Kolmer's quantitative complement-fixation test for syphilis a positive serum may give a negative reaction in a dose of 0.1 c.c. and a positive reaction of varying degree in doses of 0.05 c.c. of serum or less.

2. Such anomalous reactions may occur in the absence of natural hemolysin in the serum tested and are, therefore, occasionally due to the presence of serum constituents whose nature and mode of action are, as yet, unknown.

3. In a definite proportion of anomalously-reacting sera the reaction is due to the presence of relatively large amounts of natural antisheep hemolysin.

4. Anomalous reactions in the first tube of Kolmer's quantitative test may occur after the absorption of the natural antisheep amboceptor, and absorption of hemolysin from hemolysin-containing sera may be without effect upon the anomalous reaction.

5. In a definite proportion of hemolysin-containing and anomalously-reacting sera, while the strength of the reaction in the first tube may be increased in the first tube by the absorption of the natural antisheep hemolysin, the ultimate dilution in which the serum reacts after absorption may be affected and decreased.

6. In Kolmer's quantitative method these anomalous reactions are of no practical importance and do not necessitate prior absorption of the sera, as the strength of the reaction is not materially affected by the anomalous reactions occurring in the first tube.

7. Unknown serum constituents, not removable by exposure to sheep cells and capable of causing hemolysis, are a potential, though infrequent, source of error in the production of false negative reactions *when only a single dose of 0.1 c.c. of serum is tested* in complement-fixation methods for syphilis.

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MEASUREMENTS OF URIC ACID IN BLOOD BY VARIOUS METHODS*

By Wm. G. Lennox, M.D., and Marie F. O'Connor, B.S., Boston, Mass.

IN the eleven years which have elapsed since Folin and Denis[1] published their colorimetric method for the measurement of uric acid in blood, numerous improvements in the method have been made. These modifications have resulted in a greatly simplified technic, but the values obtained have

*From the Department of Neuropathology of Harvard Medical School. This study is a by-product of work done under grant from the Committee on Epilepsy, New York City. This also is No. 12 of a series of papers on metabolism from the Medical School of Harvard University and allied hospitals, a part of the expenses of which have been defrayed by grant from the Proctor Fund for the Study of Chronic Diseases.

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varied, the tendency being toward higher figures. By the original method 2 mg. of uric acid per 100 c.c. of blood was considered the upper limit of normal, whereas by the Folin-Wu[2] method, published in 1919, the upper limit is 3.0 or 3.5 mg.

Benedict[3] in 1922 proposed a further simplified direct method which does away with the preliminary precipitation of uric acid. Folin[4] has accepted this modification. He believes[5] that "there is scarcely place for further improvements" in the method of uric acid analysis. Benedict[3] found that his new procedure gave higher values than the Folin-Wu method. The increase of the new over the old method in 40 per cent of the determinations was from 0.6 to 2.0 mg. per 100 c.c. of blood. Discrepancies between the two methods were greatest in bloods containing low concentrations of uric acid. Brown and Raiziss[6] report somewhat similar results.

Folin[4] says that his present direct method (1922) gives values only 0.1 to 0.2 mg. higher than the Folin-Wu method. Using the new Folin procedure without modification (except that we did not find it necessary to use lithium oxalate as an anticoagulant in order to secure clear filtrates) we have measured the uric acid in the blood of a large group of patients with epilepsy.[7] We obtained values much in excess of the limits of normal as stated by Folin for this method. Table I summarizes our findings. Of the 114 determinations on blood drawn from two to five hours after the subjects had eaten, all but four gave values between 2.5 and 4.6 mg. per 100 c.c. of whole blood. Of 79 determinations made on blood drawn from patients (otherwise unselected) who had not eaten for twelve to fifteen hours, thirteen were above 4.6 mg. The average of the readings for this group was 0.5 mg. higher than the average for the nonfasting group. We have shown elsewhere[8] that prolonged starvation uniformly results in a greatly increased concentration of uric acid in the blood. It is possible that occasional small increases might occur in persons who had been without food for as short an interval as 12 hours.

None of the subjects recorded in Table I had abnormal concentrations of nonprotein or urea nitrogen in the blood, or showed other evidence of nephritis. Intensive study of a number of them revealed no evidence of faulty uric acid metabolism. As a further check on our results we measured the uric acid in the blood of 20 known normal persons, mostly students. Blood from these was obtained in the morning before breakfast. Concentrations of blood nonprotein and urea nitrogen were normal. Concentrations of uric acid varied from 3.3 to 5.7 mg., with an average of 4.5 mg. per 100 c.c. of blood. It is possible that these results were somewhat high because blood was taken under fasting conditions. The number of these determinations is too small to permit any statement concerning the normal blood uric acid limits. However, it seems probable that if Folin's new direct method is adopted, the upper limit of uric acid in the blood of normal, nonfasting individuals will need to be raised from the present level of 3.0 or 3.5 mg. to a new level of 4.5 or 5.0 mg. per 100 c.c. of whole blood.

These unexpectedly high uric acid figures led us to make parallel deter-

minations by other methods. Blood for these analyses was taken from normal and epileptic subjects before and during starvation. Table II shows the relationship in 27 instances between uric acid concentrations as measured by the Folin-Wu (1919) and the Folin (1922) methods. In all but three determinations, the values by the new direct procedure exceed those by the old method by more than 0.5 mg. Discrepancies between the two methods hold for both normal and abnormal concentrations of uric acid. There is no constancy in the amounts of difference.

Table III gives a comparison of results by the new method of Benedict and of Folin. The differences are small. Of the 30 determinations the Folin method is higher by more than 0.5 mg. in five. The average of all determinations by the Folin method is 0.2 mg. higher than by the Benedict.

TABLE I

DATA CONCERNING 193 MEASUREMENTS OF URIC ACID IN THE BLOOD OF PATIENTS WITH EPILEPSY

BLOOD TAKEN NO. OF HOURS AFTER FOOD	NO. OF SUBJECTS	NO. OF MEASUREMENTS	MG. PER 100 C.C. WHOLE BLOOD			PER CENT ABOVE 4.6 MG.
			MAXIMUM	MINIMUM	AVERAGE	
2-5	79.	114	6.0	2.5	3.5	4.
12-15	69.	79.	6.5	2.3	4.0	16.

TABLE II

CONCENTRATION OF URIC ACID IN BLOOD BY THE METHODS OF FOLIN (1922) AND FOLIN-WU (1919)

MG. PER 100 C.C. WHOLE BLOOD					
FOLIN (1922)	FOLIN-WU (1919)	EXCESS OF FOLIN OVER FOLIN-WU	FOLIN (1922)	FOLIN-WU (1919)	EXCESS OF FOLIN OVER FOLIN-WU
2.3	1.9	.4	8.2	7.2	1.0
2.9	1.8	1.1	8.3	6.7	1.6
2.9	1.9	1.0	9.1	8.5	.6
3.0	2.5	.5	9.2	8.3	.9
3.1	2.1	1.0	9.2	8.3	.9
3.3	2.0	1.3	9.5	8.7	.8
3.6	1.9	1.7	9.5	8.9	.6
3.7	3.6	.1	9.5	6 7	2.8
4.0	3.3	.7	9.7	6 7	3.0
5.3	3.7	1.6	9.7	6.7	3.0
5.4	5.0	.4	10.0	9.1	.9
5.8	5.2	.6	10.2	8.8	1.4
6.1	4.2	1.9	10.7	8.3	2.4
6.1	4.8	1.3			

Folin[9] suggests preliminary precipitation of uric acid by means of silver lactate (his check method), in order to make sure that no significant portion of the blue color is due to substances other than uric acid. Table IV gives comparative results obtained by Folin's direct and check procedures. In all determinations results by the direct method exceed those by the check, though the differences are not so great as between the direct and Folin-Wu methods (Table II). It is evident that in our hands and in the bloods with which we have worked, values obtained by use of the new direct procedures of Benedict and of Folin are considerably higher than those obtained by the longer method of Folin and Wu. We cannot attempt explanation of

TABLE III

CONCENTRATION OF URIC ACID IN BLOOD BY THE METHODS OF FOLIN (1922) AND BENEDICT (1922)

MG. PER 100 C.C. WHOLE BLOOD					
FOLIN (1922)	BENEDICT (1922)	EXCESS OF FOLIN OVER BENEDICT	FOLIN (1922)	BENEDICT (1922)	EXCESS OF FOLIN OVER BENEDICT
3.3	3.4	−.1	4.7	4.4	.3
3.5	3.5	0	4.7	4.6	.1
3.8	3.6	.2	4.8	4.5	.3
3.8	3.7	.1	5.0	4.6	.4
3.8	3.7	.1	5.7	4.7	1.0
3.8	3.6	.2	6.2	6.2	0
4.0	3.7	.3	6.3	6.8	−.5
4.0	3.8	.2	6.4	6.7	−.3
4.2	4.0	.2	6.8	7.0	−.2
4.4	4.1	.3	7.0	6.7	.3
4.5	4.0	.5	7.3	7.0	.3
4.5	4.2	.3	7.7	7.3	.4
4.5	4.6	−.1	8.2	7.0	1.2
4.6	4.0	.6	8.4	8.9	−.5
4.7	4.1	.6	10.4	9.4	1.0

TABLE IV

CONCENTRATION OF URIC ACID IN BLOOD BY DIRECT AND BY CHECK METHODS OF FOLIN (1922)

DIRECT	CHECK	INCREASE OF DIRECT OVER CHECK	DIRECT	CHECK	INCREASE OF DIRECT OVER CHECK
2.9	2.5	.4	6.2	5.2	1.0
3.0	2.8	.2	6.2	5.0	1.2
3.1	2.1	1.0	6.7	5.7	1.0
3.3	2.2	1.1	6.7	5.5	1.2
3.6	1.9	1.7	6.8	5.0	1.8
4.1	3.0	1.1	6.8	6.2	.6
4.3	3.6	.7	7.1	6.2	.9
4.3	4.1	.2	7.6	7.2	.4
4.5	4.2	.3	8.0	8.0	0
4.6	3.8	.8	8.5	7.3	1.2
4.9	3.6	1.3	8.7	7.6	1.1
5.0	4.3	.7	8.9	7.8	1.1
5.3	4.3	1.0	9.0	8.6	.4
5.5	4.3	1.2	9.2	6.5	2.7
6.1	5.2	.9	9.5	9.4	.1
6.1	4.7	1.4			

these differences, or present evidence as to which method, if any, gives correct measurement of the uric acid in blood. The use of various methods which yield discordant results adds greatly to the difficulties of clinical studies. It is to be hoped that conclusive evidence will be presented of the correctness of one or the other procedure for measuring uric acid in blood.

SUMMARY

It seems probable that the upper limit of normal for the new direct method of Folin may be 4.5 or 5.0 mg. per 100 c.c. of blood.

The concentration of uric acid in blood is considerably but inconstantly higher when measured by the direct methods of Benedict or of Folin (1922) than when measured by the method of Folin-Wu (1919) or by the check method of Folin.

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ORGANIC, PROTEIN AND COLLOIDAL SILVER COMPOUNDS*

IV. Deterioration of Solutions on Keeping

By Torald Sollmann and J. D. Pilcher, Cleveland, Ohio

IT is currently believed that solutions of the organic silver compounds deteriorate rapidly on keeping, but we are not familiar with any quantitative data for this belief; and indeed it does not appear quite clear whether the assumed deterioration is in the direction of diminution or increase of the silver-ion concentration. The former would mean a decrease of antiseptic efficiency; while decomposition of colloidal into ionic silver would increase the irritation, but would also increase the antiseptic efficiency.

Table I

Aging of Silver Solutions on their Antiseptic Activity

The figures represent the average inhibiting dose, i.e., the quantity of the compound, in milligrams per 10 c.c. of mixture, required to prevent gas formation by yeast under standard conditions.

Age of solutions:		One day	One week	One month	Two months	Four months	Eight months	One year
In water	Silver nitrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Protargol	2.2	2.6	2.7	3.1	3.1	3.5	5.0
	Argyrol	66.	42.	21.	21.	17.	25.	30.
	Silvol	93.	70.	61.	65.	60.	89.	—
In 0.9% Sodium chloride	Silver Nitrate	20	20.	20.	20.	20.	20.	20.
	Protargol	63	80	48	68	>88	>140	>200
	Argyrol	—	105	<49	29	48	37	
	Silvol	140	>250	90	84	90	>145	

*From the Department of Pharmacology of the Medical School of Western Reserve University, Cleveland.

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TABLE II

THE ANTISEPTIC EFFICIENCY OF SILVER SOLUTIONS, EXPRESSED AS PERCENTAGE OF THE EFFICIENCY OF THE FRESH SOLUTIONS

AGE OF SOLUTIONS:		ONE DAY	ONE WEEK	ONE MONTH	TWO MONTHS	FOUR MONTHS	EIGHT MONTHS	ONE YEAR
In water	Silver Nitrate	100	100	100	100	100	100	100
	Protargol	100	85	83	71	71	63	44
	Argyrol	100	157	314	314	390	264	220
	Silvol	100	133	153	143	155	104	—
In 0.9% Sodium chloride	Protargol	100	79	131	92	<71	<45	<31
	Argyrol*		100	>214	362	218	283	—
	Silvol	100	<56	155	166	155	<96	

*Expressed in percentage of solutions a week old.

With the hope of supplying direct and quantitative answers to these questions, we have applied the yeast method, to test the antiseptic efficiency, and therefore the silver-ion concentration, of solutions of representative silver compounds, kept for various periods of time.

The following solutions were used:

Silver nitrate	10%
Protargol,	10%, two specimens.
Argyrol,	10%, two specimens.
Silvol,	20%, three specimens.

The solutions were kept in ordinary bottles, cork stoppered, in diffuse daylight, without special precautions as to asepsis. The concentration re-

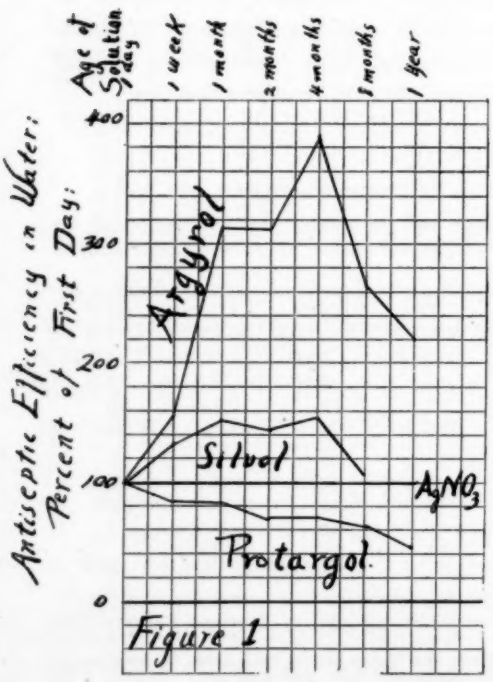

Fig. 1.

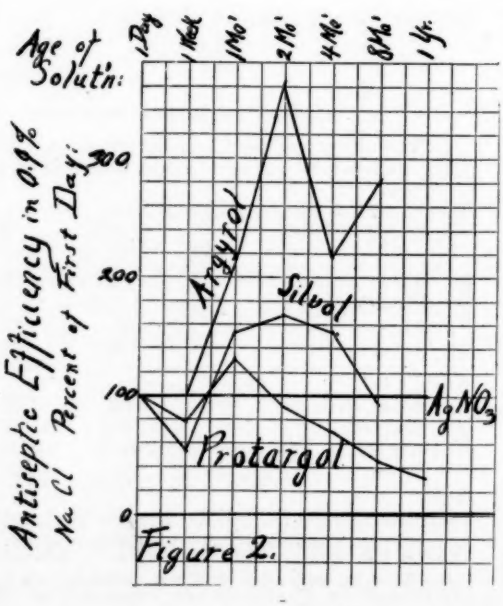

Fig. 2.

quired to inhibit the growth of yeast was determined in water, and also in normal saline, by the method described in our previous papers, on the day when the solution was made, and again at the end of a week, and of 1, 2, 4, and 8 and 12 months.

Table I shows the averages of the dosage required to inhibit the growth of yeast.

The changes will be more easily appreciated if they are expressed in terms of the original antiseptic efficiency, as in Table II and Figs. 1 and 2.

SUMMARY

The graphs show at a glance that the organic compounds undergo progressive changes, which are quite marked within a week. They are fairly parallel for watery and saline media; but their direction is absolutely the reverse for protargol on the one hand, and for argyrol and silvol on the other hand:

1. Protargol (which may be taken as representative of the *Protargentum Fortius* type) *loses* progressively in antiseptic activity. Evidently the ionic silver (which constitutes about $^9/_{10}$ of its total silver content) is gradually reduced to insoluble or otherwise inactive silver. The *activity is decreased,* in a month, to about $^4/_5$ of the original; in 8 months, to about $^2/_3$ of the original; in a year, to about $^1/_2$ of the original.

The loss, although considerable from the standpoint of percentage, would probably not have much clinical importance.

Argyrol and Silvol (representing the type of *Protargentum Mite*) *increase* in antiseptic efficiency, and therefore in irritation, with age. This is probably due to the progressive ionization of a part of their colloidal silver, perhaps through decomposition of their protective protein. The *activity and irritation are increased:* In a week, by about $^1/_2$ for argyrol, $^1/_3$ for silvol; in a month and onward, to about 3 times for argyrol, by about $^1/_2$ for silvol.

The changes within a week might cause sufficient irritation to modify the clinical response.

CONCLUSIONS

Solutions of the colloidal silver compounds show progressive changes of antiseptic efficiency on keeping.

Protargol solutions (and presumably others of the same type) become poorer in ionic silver, and therefore less efficient; but even in a year the change would not be of much clinical importance.

Argyrol and Silvol (and presumably others of the type), become richer in silver ions, and therefore more antiseptic, but also more irritant. The changes start rapidly, so that a week might suffice to modify the clinical response.

STUDIES IN BLOOD CELL MORPHOLOGY AND FUNCTION*

II. Morphologic Changes of the Blood in Myelogenous Leucemia under Radium Treatment

By Max Maurice Strumia, M.D., Philadelphia, Pa.

SINCE the work of Renon[1] and his coworkers in France, and Schueller[2] in Germany, the study and application of radium in the treatment of leucemia, especially of the myelogenous type, has been extensive; and, as is most widely admitted today, it constitutes the best known therapeutic agent against this disease.

The changes occurring in the circulating blood during radium treatment are of utmost importance, because they reveal, to a certain extent, the modifications which are going on in the foci of the leucemic tissue. The results and conclusions of some of these studies will be considered later. The purpose of this work is to present an accurate study of the changes occurring in the circulating blood of a patient of myelogenous leucemia during the radium treatment, together with some new observations on the relative behavior to radium of the various blood cells, in order that data may be gained to corroborate with biologic proofs the relationships of some of the blood cells.

Studies of such a nature have been rather numerous, yet the lack of detailed differential counts in some of them and irregular and perhaps inaccurate blood counts in others have vitiated the results of these interesting studies. Especially faulty has been the frequent massing of cells of entirely different origin, as primordial cells, myeloblasts, large lymphoblasts, etc., under the common name of mononuclear cells.

From an extensive review of the literature on the subject, it seems that roentgen rays and radium are fundamentally alike in their action on the leucemia, provided that the same quality of radiation is used. Radium is perhaps more constant and quicker in its effect (Gulland,[3] Ordway,[4] etc.).

Falta and his coworkers,[5] confirmed by Kauffman,[6] found that thorium and mesothorium are similar to radium in their effect on leucemia.

Various findings of investigators using roentgen rays will be promiscuously referred to, as well as findings of those using radium in any form or method.

Case.—The patient, J. A., age forty-eight, white, male, has no points of interest in the family history, and in his remote anamnesis. About one year and a half prior to the time when first seen, the patient noticed a lump just below the margin of the ribs on the left side, which gradually increased in size. The patient did not feel any other

*From the Department of Pathology and Bacteriology of the Graduate School of Medicine of the University of Pennsylvania and the Laboratory of Clinical Pathology of the Philadelphia General Hospital.

The first paper of this series—"Reticulosis; Increased Percentage of Reticulated Erythrocytes in the Peripheral Blood"—appeared in the Jour. Lab. and Clin. Med., 1922, viii, 11.
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serious symptoms referable to the disease, except a vague sense of weakness, pallor, and loss of appetite.

The first physician from whom the patient sought advice administered a few intravenous injections of an arsenical preparation. The patient first came to our notice in the beginning of October, 1922, and at that time showed a rather high degree of anemia; the heart was slightly enlarged, and the first sound was accompanied by a very light, soft, blowing murmur. The blood-Wassermann was negative. The spleen reached five inches below the costal margin, and the liver was also palpable two and one-half inches below the costal margin. The blood count was: White cells—133,000; R. B. C. 4,450,000, Hb. 75. The differential count is given in Table III. This count is the average of three counts taken on three consecutive days, the last being taken October 10, 1922. The following day, radium treatment was started at the Radiological Department of Philadelphia General Hospital under the direction of Dr. R. Bradley, to whom I am greatly indebted for his valuable cooperation and for the account given as to the method of the radium applications.

In doing the blood examinations, the following precautions were used: the samples for blood counts were taken with the same pipettes, certified, and the counts were done with the same counting chamber, also certified, using the same technic, the same person doing all the counts.

The Hb. estimations were done with the colorimeter, using as a standard (100 per cent) a solution of dog's blood in N/10 HCl., containing hemoglobin in amount of 13.8 per cent. The preparation of the standard was done by determining the oxygen capacity of the dog's blood.

The slides were stained with May-Giemsa stains, using Pappenheim's technic,[7] and with the oxidase stain, using Goodpasture's[7a] method. The methyl-green-pyronin method was also employed in the differentiation of lymphatic cells. The differential counts were made on two separate slides, at different times, to avoid bias and self-suggestion, not less than 500 cells being enumerated. As a matter of fact, in 33 differential counts made, 24,846 cells were counted, averaging 753 cells at each enumeration. Enough emphasis cannot be laid upon the necessity of faultless technic in blood sampling, in order that the results be strictly comparable. The necessity of counting a very large number of cells is just as important, so as to obtain a true figure, especially when the variety of cells is large.

In recording differential counts, Ferrata's[8] classification, somewhat modified, was used. The blood counts were taken for the first few months possibly every other day, always in the morning before the radium application. After the first two months, at variable periods of time as indicated in Table I and III.

The coagulation time was taken approximately every month. The radium was applied in form of emanations, using the method of pack, the radiating tubes being at a distance of six inches from the skin and filtered so as to leave free course to the penetrant gamma rays. Table IV gives details of the radium applications.

We will now analyze the blood picture following radium application.

White Cell Count, Red Cell Count and Hemoglobin.—It will be seen from Table I, that from the first application or two, both leucocytes and erythrocytes greatly increased in number; the white cells from 133,000 to 202,700 and the red cells from 4,450,000 to a maximum of 7,040,000. This considerable increase is without doubt due to the stimulation or irritation of the first doses of radium, as was confirmed by changes in the differential count and by the very peculiar increase of the nucleated red cells, especially of the megaloblastic type, as will be discussed later.

The white cells, after the first momentary rise, steadily decreased under the effect of radium; then they oscillated for about a month between 17,000 and 40,000 and finally went down to normal. The downward course of the white cells continued also after the radium applications were discontinued, reaching a minimum of 5,000 about three months after the last dose of radium. This indicates the advisability of stopping the radium application as soon as the white cells are near normal, between 10,000 and 15,000. Gulland[3] also found that the effect of radiation goes on for a while after the termination of the treatment.

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The last applications of radium were given on December 20, 1922, the white cells numbering about 6,000. They started to increase again in June, 1923, six months later, and then they rapidly increased, reaching 36,000 at the end of July, 1923, and 75,000 on August 29, 1923.

The red cells after the temporary increase were no longer affected, so far as the count is concerned.

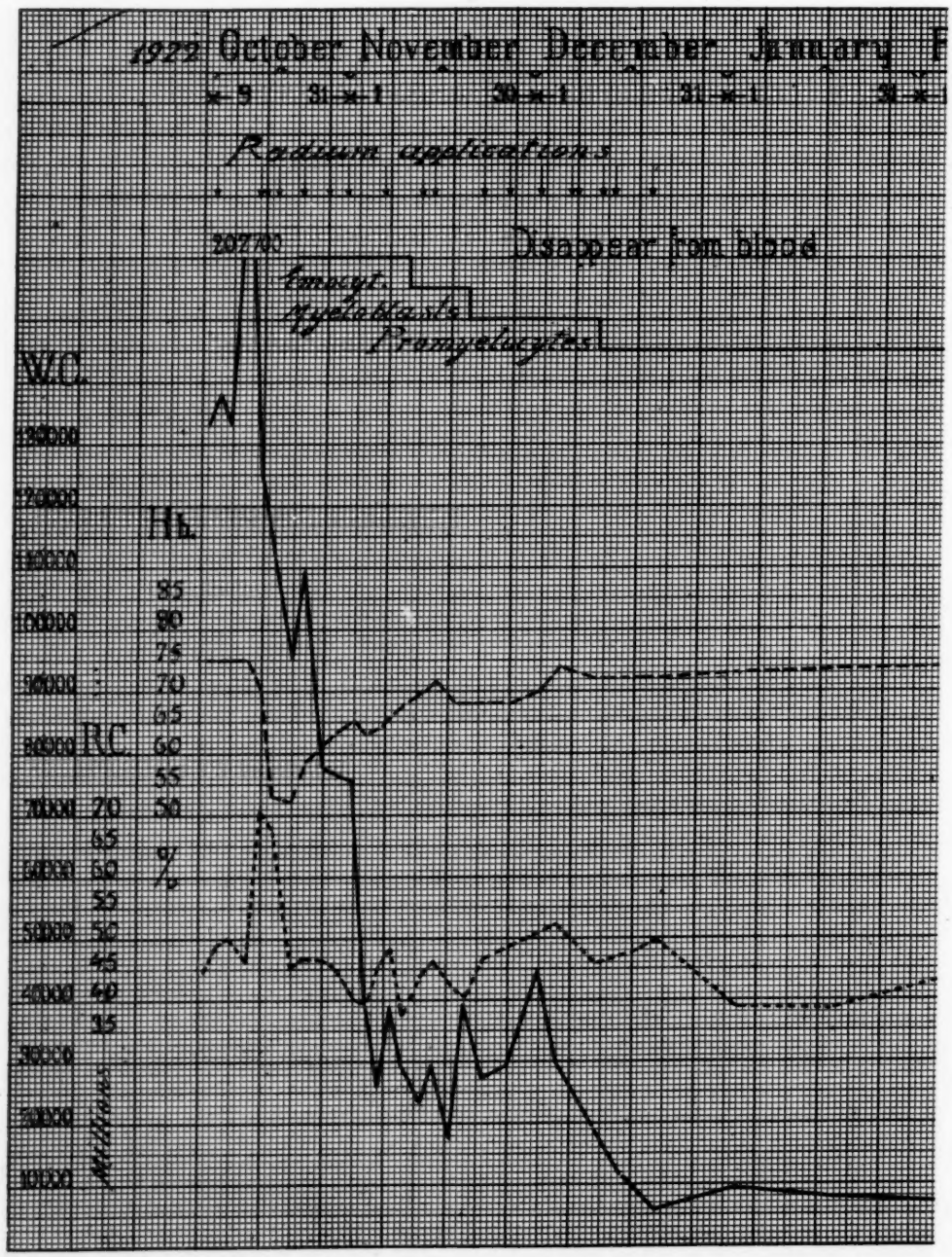

Table I.—Variations of the blood counts and the hemoglobin percentage; this diagram also shows order in which immature cells disappear from the blood. The radium applications are recorded in the upper portion.

The hemoglobin, during the period of rapid increase of red cells, decreased from 75 per cent to 52 per cent in seven days, which testifies to the irregular production of red cells under the stimulus of radium. After this drop, the hemoglobin slowly rose, reaching a percentage of 85, as compared to 75 per cent previous to treatment.

During the last month of observations, August, 1923, the hemoglobin decreased to 70 per cent again, while the leucocytes rose from 26,000 to 75,000 in twenty-nine days.

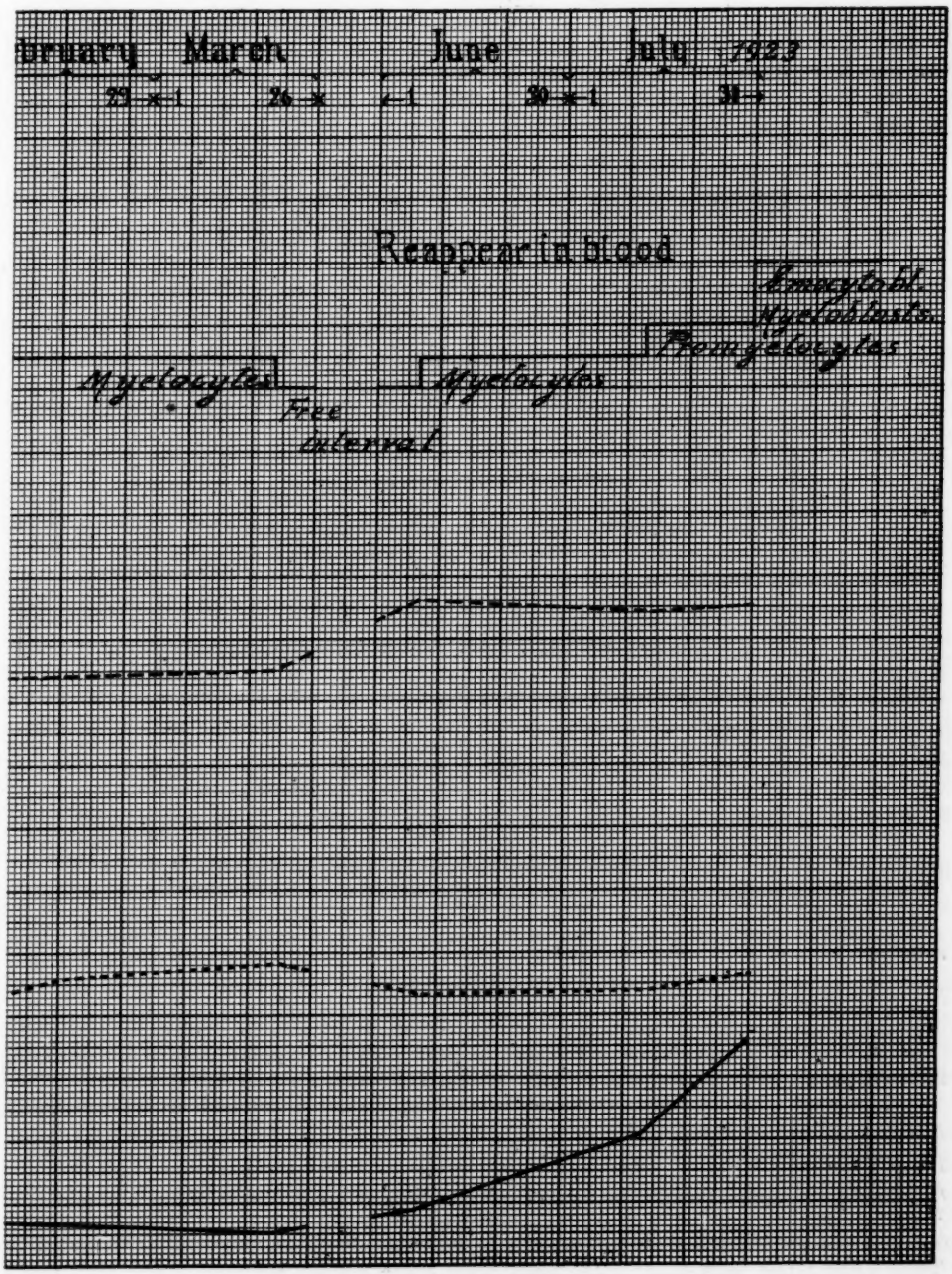

Table I—Cont'd

It is therefore obvious that the curves of the white cell counts and the percentage of hemoglobin run an exactly contrary course.

Primordial Cells.—The primordial cells, or hemocytoblasts (Ferrata[8]), which should be identified with Pappenheim's lymphoidocytes, after a short period of stimulation in which they were slightly increased, rapidly and steadily decreased until they totally disappeared a month after the beginning of the treatment, or after ten radiations had been given, totalling 46,400 millicurie hours.

The order in which the immature cells disappeared from the blood remarkably bears out their genealogic relationship; in fact as it is shown in the upper part of Table I, the first cells to disappear were the primordial cells, which in all probability are the undifferentiated mother cells of all the cellular elements of the blood; then the myeloblasts; the promyelocytes and finally the myelocytes, all belonging to the granulocytic series.

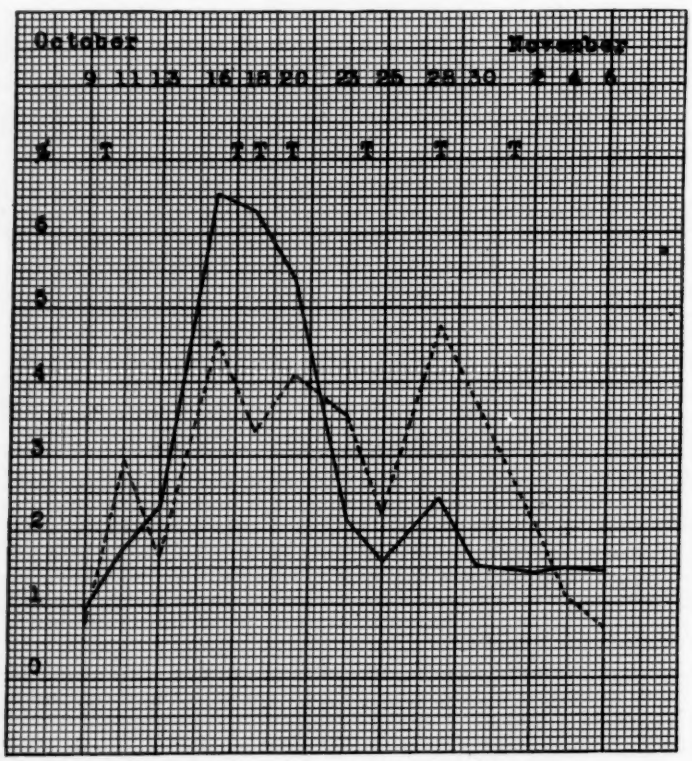

Table II.—The stimulating effect of the first radium applications on the immature and pathologic cells of the hemoglobinic series.

———: Pathologic cells of the hemoglobinic series.
.....: Immature cells of the hemoglobinic series.
T: Radium treatment.

In contrast, the primordial cells were the last cells to reappear when the white cells increased again, six months after the last radium application.

Immature Cells of the Leucocytic Series.—Myeloblasts, promyelocytes and myelocytes disappeared in the order mentioned. The metamyelocytes were the only cells of the series present, in small number, after the radium treatment, which testified that the pathologic activity of the leucemic centers was only greatly attenuated, not entirely destroyed by the radium. The period of time in which immature cells of the leucocytic series were almost absent from the blood and in which red cells and hemoglobin were normal is indicated in Table I, as "free interval." This lasted about three months, starting about seven weeks after the last radium treatment was given. During this interval the size of the spleen was within normal limits and the patient's general condition good.

Several blood counts and differentials were conducted during this period, but the variations were of no importance and are not reported in Table I. During the period of treatment with radium, the relative number of mast cells was apparently increased, (Whitcher[9]).

The considerably longer time required by the myelocytes to disappear from the blood as compared with the other immature cells of the granulocytic series (myeloblasts, promyelocytes), is due to the fact that the myelocyte is an adult form which in order to become a normal polymorphonuclear does not require anything but an ontogenetic ripening; (Ferrata[8]). In other words, as to the effect produced by radium, the granulocytic cells are sharply divided in two classes; cells having totally (myeloblasts) or partially (promyelocytes) basophilic protoplasm, which are readily affected by radium, and cells with neutrophilic protoplasm (myelocytes, metamyelocytes, polymorphonuclears) which are less easily affected by the radium.

Myeloblasts, and promyelocytes are, as a matter of fact, the cells whose presence in the circulating blood, together with other symptoms, has an almost pathognomonic value for the dignosis of myeloid leucemia, because these cells are very seldom found in other conditions, while small numbers of myelocytes are found in many other diseases, when the hemopoietic organs are more or less involved.

The effect upon the immature cells of the lymphocytic series will be considered later.

Immature Cells of the Hemoglobinic Series.—During the days following the first two radiations, the number of the nucleated red cells increased considerably, together with the number of the pathologic red cells as shown by Table II. This increase, evidently due to a stimulation from the radium, is even more interesting considering that it acted to a great extent upon the megaloblastic elements which were considerably increased after the first application. In the blood smears taken those days, the number of megaloblasts and normoblasts in mitosis was surprising, a sign of great activity in the erythropoietic centers.

Following this rise, the number of the nucleated red cells rapidly decreased under the effect of large doses of radium, the megaloblasts being the first to disappear, adding another point to the theory that the less mature the cells, the more quickly they are affected by radiation. Nucleated red cells of all kinds and pathologic red cells entirely disappeared during the so-called "*free interval,*" to reappear with the renewed activity of the hemopoietic tissue, when the white cells increased again in number. This similar behavior of the red and white cells bears out the relationship between affections of the erythropoietic and of the leucopoietic system. Perhaps an illustration of this is found in the disease, or better syndrome, known under the name of Von Jaksch's,[10] or pseudoleucemic anemia of childhood.

Pathologic Cells of Leucocytic Series.—These were also increased slightly, absolutely as well as relatively, after the first few doses of radium, especially the Türk and Rieder cells, (degenerative forms) but soon under the effect of subsequent doses of radium, they entirely disappeared from the circulation. (See Table III.)

Platelets and Coagulation Time.—In another case of leucemia being treated with radiation, we noted the increasing facility with which the blood would flow from the finger tip as the treatment was being given. In this case, this phenomenon was also noted. The coagulation time, which was taken every month or so, before treatment was about two minutes. During the treatment the coagulation time steadily increased and was six minutes during the "*free interval*"; then during the last two months of observation, while the white cells were going up again, the coagulation time was reduced to three and one-half minutes. The platelets and their progenitors, the megakaryocytes, followed exactly the same course: the megakaryocytes disappeared from the peripheral blood after eight applications of radium in a period of twenty days, and the platelets, which were very numerous in the first blood films, diminished considerably until they were reduced to normal number in about two months after treatment was begun. Giffin[11] has also reported diminution of the number of platelets following protracted radiation treatment.

Mature Leucocytes.—The absolute count of the polymorphonuclear neutrophiles went steadily down from 73,788 per cmm. before the treatment, to a minimum of 2,761 per cmm. during the "*free interval,*" to climb again during the last two months. The relative count, however, increased during the treatment from 55.48 per cent to a maximum of 86.04 per cent, which was reached when 19 treatments of radium out of the total of 20 had been given.

The relative count of the polymorphonuclears stood about 80 per cent during the last period of the treatment, while the absolute count of the mature lymphocytes was around 800 per cmm. and their relative count was about 10 per cent.

A few days after the treatment of radium was stopped, the relative count of the

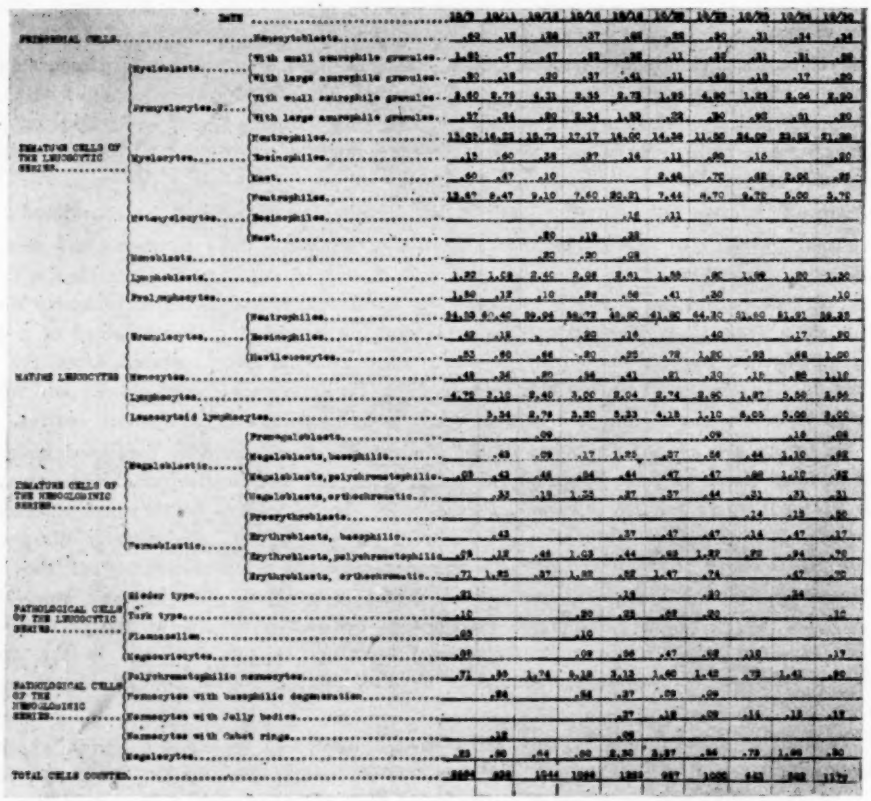

Table III.—Differential counts taken through the period of observation.

polymorphonuclears started to diminish, reaching a minimum of 55.22 per cent, with an absolute number of 2,761 on March 20, 1923, during the "*free interval.*"

The lymphocyte count, both absolute and relative, steadily climbed after the treatment was stopped, and on March 20, their relative count was at the maximum of 37.23 per cent, while the absolute number was 1861 cells per cmm., that is a little over the normal.

Summarizing: during the treatment the polymorphonuclears diminished in the absolute number, but their relative number went up; lymphocytes diminished in the absolute and relative number. Immediately after treatment, the number of polymorphonuclears, both relative and absolute, went down; while the lymphocyte number, both absolute and relative, went up. This tends to show that apparently lymphocytes pick up very easily as soon as the radium treatment is discontinued, while the radium action on the mature granulocytes goes on for a longer period. This point will be further discussed later.

Perhaps the most striking effect of the radium upon the circulating lymphocytes, was the pouring out in the blood stream, following the first radiation, of a very large number of lymphocytes which were on the borderline between normal and pathologic cells. At any rate they are partially degenerated cells, which can be identified as the leucocytoid lymphocytes of Pappenheim (see picture). They are cells of large dimension, from 18 to 20 microns, much larger therefore than the common lymphocytes, with a relatively large band of protoplasm, which hardly appears to be stained, so that the margins of the cells are sometimes hard to make out. The nucleus has the usual characteristics of the lymphocytic nucleus, but it is even darker and somewhat pyknotic; the masses of chromatin almost forming a solid mass. The protoplasm may or may not contain a variable number, sometimes very large, of the usual azurophile granules.

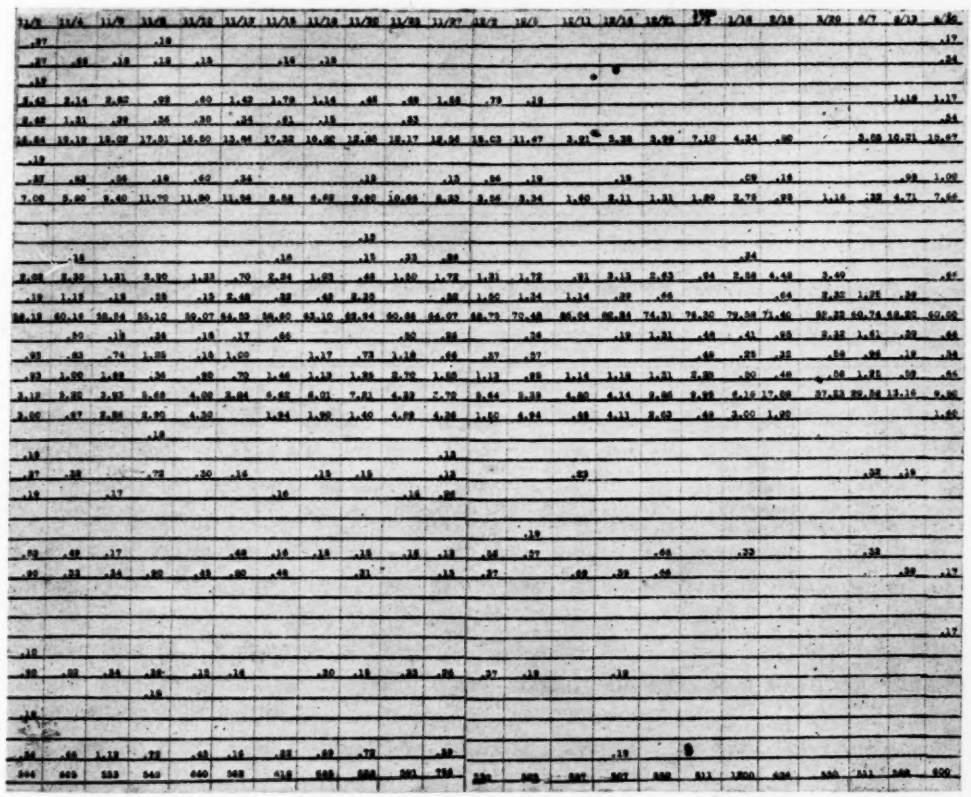

Table III—Cont'd

Pappenheim gives to the leucocytoid lymphocytes, the value of old lymphocytes; in the case presented, their presence was with all probability caused by the destructive action of the radium upon the lymphoblastic centers. Such forms disappeared almost entirely during the *"free interval."*

Mononuclear cells were considerably reduced in number during treatment, their absolute number slowly decreasing from 532 before the treatment to considerably below the normal. The relative number increased during the treatment, reaching a maximum of 2.28 per cent.

The Spleen rapidly diminished in size and before the treatment was over it was no longer palpable. This agrees with the findings of all investigators in this field.

Symptomatic conditions were characterized by marked clinical improvement during treatment, with considerable gain in weight.

TABLE IV

RADIUM APPLICATIONS

DATE	MC. HOURS	LOCATION
10-10-22	4,658	Lower end, right leg.
10-10-22	4,658	Upper " " "
10-17-22	4,656	Lower " left leg.
10-18-22	4,700	Upper " " "
10-20-22	4,640	Lower " right femur.
10-24-22	4,655	Upper " " "
10-28-22	4,500	Lower " left "
11- 1-22	4,730	Upper " " "
11- 7-22	4,500	Lower " " forearm.
11- 8-22	4,563	Upper " " "
11-13-22	4,488	Lower " right "
11-15-22	4,500	Upper " " "
11-23-22	4,500	Lower " " "
11-27-22	4,680	Lower " " arm.
12- 2-22	4,636	Upper " " "
12- 7-22	2,594	Upper " left . "
12- 8-22	2,140	Lower " " "
12-12-22	3,903	Upper thoracic vertebrae.
12-14-22	4,038	Lower " "
12-20-22	4,476	Lumbar vertebrae.

TABLE V

SHOWING VARIATIONS OF THE ABSOLUTE NUMBER OF POLYMORPHONUCLEARS AND LYMPHOCYTES PER C.MM.

DATE	POLYMORPHONUCLEARS	LYMPHOCYTES
10- 9-22	73,788	4,251
10-11-22	84,345	11,647
10-13-22	80,624	6,862
10-16-22	118,998	12,567
10-18-22	57,300	9,200
10-20-22	70,022	7,772
10-23-22	63,605	3,705
10-25-22	56,981	8,691
10-28-22	41,073	8,174
10-30-22	45,555	5,019
11- 2-22	45,170	4,608
11- 4-22	24,288	2,300
11- 6-22	15,019	1,278
11- 8-22	22,048	3,324
11-10-22	17,217	2,407
11-13-22	15,197	656
11-15-22	17,333	2,508
11-18-22	11,247	1,085
11-20-22	25,513	3,383
11-23-22	16,948	2,514
11-27-22	18,847	2,337
12- 2-22	30,896	3,191
12- 5-22	21,393	2,847
12-11-22	16,347	1,000
12-16-22	9,891	990
12-21-22	4,461	736
1- 3-23	6,999	944
1-18-23	6,258	714
2-15-23	4,774	1,209
3-20-23	2,761	1,861
6- 7-23	5,507	2,573
7-13-23	14,168	2,710
7-30-23	21,960	4,377

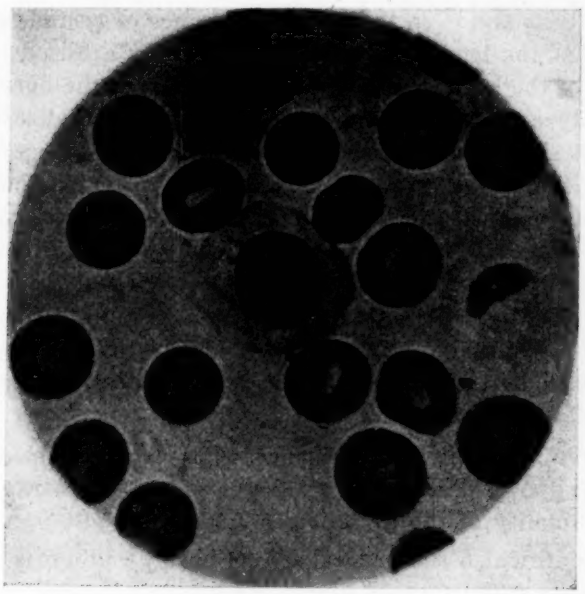

Fig. 1.—Photomicrograph of a leucocytoid lymphocyte, (x1120).

DISCUSSION

The results of this study are so emphatic as to suggest an explanation of the mechanism of the action of radium emanations upon the leucemic foci. The literature on this subject apparently rules out the spleen as playing an important part in this process.

Gulland[3] applied radium on the long bones and asserted that in no case is the starting point of the disease in the spleen. The successful results obtained from radiating the spleen alone are probably due in the opinion of the author to the fact that the organ is extremely vascular, and that a large part of the blood must pass through and thus be exposed to the radiation. Renon and Degrais, Giffin, Whitcher, already mentioned, Peabody,[12] and Shisler[13] applied radiations over the spleen. Oppenheimer[14] radiated, in one case, spleen and liver: Ordway[4] radiates the spleen, and does not consider it necessary or advisable to radiate the long bones.

Renon, Degrais and Tribaut[15] treated a patient of myeloid leucemia, whose spleen had previously been taken out, with radium applied in the region where the spleen used to be. The results were the same as those obtained when the radium was applied to the spleen. The final conclusion is that the spleen is secondary in the leucemic process. The same conclusion is reached by Giffin[16] after experimenting with splenectomy in leucemia.

Renon and his coworkers[15] believe that either radium acts on all the tissue exposed, or only on the circulating blood. This last view sustained by Gulland is admissible, in a measure, but does not explain the fact that the changes in the blood are relatively permanent and that they must therefore presuppose changes in the leucemic foci.

Radical changes were obtained in the case here presented, both in the

total number of cells and even in the disappearance of certain groups of cells. If we assume that the leucemic process is uniformly diffused in the body or at least in most of the hematopoietic centers, that is, in the bone marrow, then by radiating only a portion of the leucemic tissue, a localized and not generalized reaction should be obtained. Or, stated in another way, a quantitative and perhaps qualitative variation might be expected, but not the disappearance of all the cells of a certain type, and this was exactly the case, because long before all the bones of the lower extremities were entirely radiated, leaving untouched all the rest of the skeleton, the primordial cells disappeared entirely from the circulating blood. Therefore it is a reasonable assumption that radium acts upon the leucemic tissues in a generalized manner. Russ[25] found that doses of radium applied locally in malignant cases had a general effect on blood.

It is apparently of little significance where the radium is applied, because the entire body shares a large amount of penetrant rays; of most importance is the quality of the radiations.

The manner in which the generalized action by radium is produced, does not lend itself to a definite conclusion. We offer as a possible explanation a production of leucotoxic material by the disintegration of the cells directly affected by the radiations, in the circulating blood, or in the leucemic foci of the region radiated. This material, circulating in the blood stream, may be the cause for such a generalized alteration of hematopoietic centers. It may be possible that the blood cells and blood plasma, perhaps, carry the radium emanations from the point of application to all the leucemic centers, thus bringing about the destructive changes. We offer these explanations merely as possibilities.

That limited doses of radium produce a stimulating effect on all hemopoietic centers is borne out by the increase in the number of leucocytes and erythrocytes; and by the increase in the number of immature (nucleated) red cells, especially of the megaloblastic type, observed after the first few radium applications. The same results were observed in two other cases of myelogenous leucemia, treated with radium. Such stimulating action is of the greatest importance, because it might be made the base of the treatment of primary anemia by the use of small doses of radium.

Gulland,[3] in England, did not derive satisfactory results from radium applications in pernicious anemia, but admitted the possibility of utilizing the stimulating effect of radiations with the exclusion of the destructive. Otto Neu[17] favorably treated pernicious anemia with x-rays. Stúdying the table accompanying the report of the case of leucemia treated with radium by Whitcher,[9] it appears that following the first radium application, the white cells that were 164,800 before treatment, after a momentary drop to 135,000, increased to 312,000. Lazarus Barlow,[18] experimenting on rats, cats, rabbits, and frogs, found an increase of polymorphonuclears and erythrocytes, as a result of what he calls shock radiation. Barlow in animals[18] and Otto Neu[17] also found an increase of red cells in pernicious anemia patients under the effect of radiations. An increase of hemoglobin and erythrocytes

of leucemic patients submitted to protracted radium treatment has been observed by most of the authors who studied the subject. Such an increase is always parallel to a decrease in the number of the white cells.

Whether this increase is due to a stimulation of the erythropoietic tissue, or simply to a betterment of the conditions of lodging of the erythropoietic nests in the bone marrow, due to the reduction of the granulocytic elements, or to both, as we believe, is not easily understood. Gulland[3] puts the same question but is unable to answer it. Increase in the number of nucleated red cells following radiation is reported by Peabody[12] and a study of the tables of Schisler[13] shows that the same thing happened in his case. The stimulation of erythropoietic tissue by radium is especially marked in the megaloblastic or embryonic centers. Otto Neu,[17] treating cases of pernicious anemia, found megaloblasts in the blood which had not been present before the treatment. Price-Jones,[19] studying the effect produced by radiations in the bone marrow by exposing rabbits to five grams of radium bromide, found a definite increase in the percentage of the megaloblasts, and metrocytes. The author regards the result as due to a combination of white cell destruction, and diminished formation of granular leucocytes, with an exaggerated production of red cells. Furthermore anemia produced by radiation is often of the primary or pernicious type with megaloblasts (Gulland,[3] Giffin[11]). Cases of fatal anemia from radium are reported by Mottram,[20] Bordier[30] and others. All cases were of the aplastic type, accompanied by considerable leucopenia.

A much argued point is whether the lymphocytes of the circulating blood are more affected than the mature granulocytic cells by radium. Heinecke,[21] as the result of experimental studies, was the first to report an eclectic action of radiation upon the lymphoid tissue of the spleen, lymph nodes and bone marrow. Murphy and Ellis[22] found that by carefully regulated doses of x-ray repeated at intervals, gradual atrophy of the lymphoid tissue may be accomplished, without any appreciable effect on the other tissues.

Mottram[23] concludes from his experiments, on rats, with gamma radiations that bone marrow is less affected than lymphoid tissue. This was found true also for soft x-rays and beta rays of radium by Mottram and Russ.[24] Russ,[25] experimenting on patients suffering from malignant diseases where the body was sharing to a large extent the gamma rays, observed reduction in the number of circulating lymphocytes in twenty out of thirty-one cases studied, increase in five and no changes in six. Anything less than an 8 per cent variation was classified as no change. The figures for the polymorphonuclears in the same thirty-one cases, showed that an increase, a decrease, and no change in their number, occurred in almost the same amount. In another study, Russ and his coworkers[26] came to the almost paradoxical conclusion that the lymphocytes in the circulating blood of the rat were more sensitive to x-rays than a photographic plate. Mottram[27] found a drop in lymphocytes and polymorphonuclears in x-ray workers. The number of lymphocytes and polymorphonuclears is low in radium workers also. The

diminution in the polymorphonuclears is more marked than in x-ray workers.

Lazarus-Barlow,[18] in his experiments with rats, using five grams of radium, found that the destruction of lymphocytes is the most characteristic effect. Warthin,[28] in an experimental study on the effect of x-ray upon blood-forming organs, found a marked effect upon the lymphoid tissue of the spleen and lymphoid glands, the small lymphocytes being the first to be affected. In contrast with the finding of Russ, Mottram and the others on the subject, great reduction of lymphocytes in rats after a short exposure to radiations is not the experience and conclusion of Leitch.[29] The point was made that it is not the specific action of the x-ray, but rather the effect of the fright suffered by the nervous rat on the exposure to the noisy x-ray room. The author also failed to find any reductions of lymphocytes in the blood of five patients who were undergoing x-ray treatment.

Ordway[4] reports relative lymphocytosis after treatment; in one case reported the number of polymorphonuclears increased from 60 per cent to 78 per cent, and then diminished to 50 per cent. The absolute number fell from 299,475 to 2,917. The relative number of lymphocytes rose from 2.3 per cent to 45.7 per cent, and the absolute number of lymphocytes fell from 11,750 to 2,650. Peabody[12] found after treatment a larger proportion of mature polymorphonuclears. In one of the cases reported by Renon, Degrais and Tournemelle,[31] it is interesting to see that, notwithstanding the great reduction of white cells, the total number of lymphocytes per cubic millimeter was still 941 proportionately higher than the number of polymorphonuclears, 1,705 per cubic millimeter. Schisler[13] found a continuous relative increase of polymorphonuclears under x-rays and benzol, and a decrease in the absolute number of small lymphocytes, but no effect upon the relative count.

Whitcher,[9] in reporting a case of leucemia treated with radium which ended fatally, states that in the final period the polymorphonuclears had fallen to 4 per cent, while the lymphocytes had risen to 58 per cent. The blood picture resembled that of lymphatic leucemia. In one of the cases reported by Oppenheimer,[14] treated with x-rays, death occurred with a transition of the myelogenous leucemia into an acute lymphemia.

In our case the reduction of the absolute number of the lymphocytes was proportionately much smaller than the reduction of the polymorphonuclears, the latter being reduced to approximately one-twenty-fourth of the original number, the former only to one-sixth. Giffin[11] found that radium caused an increase in the percentage of small lymphocytes, after the reduction of the total leucocyte count; but a reduction of the absolute number to approximately one-tenth of the original number of cells.

The leucocytoid lymphocytes, which we found to be markedly increased after radium applications, may be considered in the light of our findings as partially degenerated lymphocytes. They are probably similar to those found by Warthin[28] in the peripancreatic and mesenteric lymph nodes of mice submitted to x-rays and in the spleen and lymph nodes of a patient of leucemia treated with x-rays.

A marked difference is referable to the immature cells of each series; the immature cells of the granulocytic series (myeloblasts, promyelocytes, myelocytes and metamyelocytes) are more readily and decidedly affected than the immature cells of the lymphocytic series (prolymphocytes and lymphoblasts). The lymphoblasts and prolymphocytes therefore act more like mature cells and are considerably more resistant to the radium action. This probably explains the less effective results obtained in chronic lymphatic leucemia when treated with radiations.

In a case of chronic lymphatic leucemia submitted to few radium applications, the number of small lymphocytes, lymphoblasts, and prolymphocytes was proportionately diminished in the same way. This result apparently contrasts a little with the supposition that lymphoblasts and prolymphocytes are to the lymphocytes what promyelocytes and myelocytes are to the polymorphonuclears (Ferrata[8]). In other words, geneaologically speaking, as far as the radium effect is indicative, a mature lymphocyte is nearer to the prolymphocyte and lymphoblast than a polymorphonuclear is to the myelocyte, promyelocyte and myeloblast. Summing up, the circulating lymphocyte of patients of leucemia under radiation treatment has been found by most authors to increase in percentage and to diminish in the absolute number.

In our case, the latter show a considerable and constant decrease, but stated in terms of percentage an increase occurred in the first part of the treatment and was followed by a decrease which continued even after the radium was discontinued. The experiences of Heinecke, Murphy and Ellis, Russ, Mottram, etc., on rats, reported before, have met with general approval, the only dissension being on the part of Leitch. At any rate, before any far-reaching conclusions are drawn from such reported sensibility of the circulating lymphocytes of rats to radiation, as to the mechanism of lymphocytes in the protection against diseases (Murphy and Ellis,[22] Murphy and Morton[32]), the problem should be further studied in the human being.

The order of disappearance of abnormal cells from the blood stream bears a direct relation to their immaturity or, expressed in other words, the more embryonic the cell, the easier it is affected by radium. This has been the experience of most experimenters in similar studies. We consider this phenomenon evidence which supports the conception of the histogenesis of the granulocytes from the undifferentiated or primordial cells; and of each granulocyte springing from the cell immediately above it in the granulocytic series. Further evidence of this is offered by the recurrence of the immature cells in the circulating blood (when radiation has been discontinued) in inverse manner to their disappearance. This behavior opposes the assertion of Gulland[3] that radiations have much less effect on tissues of embryonic or undifferentiated type, than on those more fully developed. Ordway[4] found the immature forms to be more affected by radium than the mature ones; in Peabody's [12] experience, under radium treatment, myelocytes and immature forms became less prominent. All this goes to corroborate the view that the more immature the cell is, the less resistant it will be to the destructive effect of radium.

It is imperative, as emphasized by Peabody and others, that the patient under radium treatment receive constant supervision. Periodic white cell count should be made, not only during radiation, but particularly after radiation, because the fluctuation in the number of leucocytes is usually parallel to the variation of the general conditions of the patient, and sometimes precedes them.

The results here presented were confirmed in every point by the study of another case of chronic myelogenous leucemia, L. R. J., an adult negro, treated with radium in exactly the same manner. In this case, which was more advanced than the other just reported, the white cell count was 390,000 on February 3, 1922. The radium treatment was started on February 4, 1922, with the same technic indicated in the other case. The first series of treatment, 14 applications with a total of 76,516 Mc. hours, ended on March 12, 1922; on March 14, the white cell count was 42,000. On March 24, the white cell count was 10,600, this further drop in the number of leucocytes confirming the view that the action of the radium goes on for a while after its applications have been discontinued. The white cell count stood near normal for about a month and then slowly rose and was 30,400 on June 11. A new series of radium applications was started on June 16, this time numbering eleven, with a total of 45,616 Mc. hours, the last application being given on September 5, 1922. This new series of applications again brought the number of white cells near normal and they were 11,700 on November 20, 1922. These results were obtained from the record cards of the patient. In the beginning of February, 1923, we started a series of more detailed blood counts on the patient. On March 14, the white cell count was 73,100; on the seventeenth a radium application of 7,416 Mc. hours was given, and on the following day the number of white cells was 116,210. In the blood films taken on that day the nucleated red blood cells were very numerous, especially those of the megaloblastic type. This confirms that the action of the first dose or doses of radium have a decidedly stimulating action upon the blood-forming centers. The treatment was then continued, but the effect of this new series of treatment on the leucocytes, although showing fundamentally the same tendencies, was considerably less marked, probably due to a certain degree of resistance gained by the leucopoietic tissue against the radium.*

The reduction of myelocytic cells in the circulating blood, both relatively and absolutely, is perhaps the most constant and important effect of the radium treatment, because it runs parallel to the reduction of the total white cell count, the gradual reduction of the spleen and to the betterment of the general condition of the patient.

The early experimenters, as well as many others, report this striking reduction of the myelocytes after radium treatment (Renon, and Degrais, Giffin, Ordway, Peabody, Schisler). Whitcher also reports a very large diminution of the myelocytes, and besides in studying his tables, one notes a *"free interval"* similar to that studied here. The last treatment of the third series of radium applications was given to his patient September 9, 1920.

*Follow up note: The patient died September, 1924, the autopsy amply confirming the diagnosis.

Myelocytes were then present. They disappeared at a later date, without new radium applications, and they were not present October 7, but they reappeared on October 28, 1920. The same thing was repeated during other periods of the treatment.

CONCLUSIONS

1. The effect on the morphology of the blood of radium emanations applied upon the long bones in two cases of chronic myelogenous leucemia has been studied.

2. The mechanism of action of radium upon the leucemic foci seems to be a complex one as if it is of a generalized as well as localized nature, therefore presupposing the intervention of some intermediate means of action, probably leucotoxins, liberated from leucocytes directly affected by radium, or the carrying of the radium emanation on the blood stream.

3. Radium acted first in these cases, especially if applied in small doses, as a stimulant, on both leucocytes and erythrocytes. On the erythrocytic series, the stimulating effect was especially evident on the cells of the embryonic type (megaloblasts).

4. Following the period of stimulation, radium manifested its destructive action especially on the leucocytes, the order of their disappearing being in direct proportion to their immaturity. Platelets were also greatly reduced by radium, and the coagulation time was proportionately lengthened.

5. There was a *"free interval"* following the radium treatment during which the morphology of the blood remained near normal, and the clinical symptoms of the disease were quiescent.

6. In their reappearance in the blood stream, the immature cells followed exactly the opposite order of their disappearance, the most immature forms being the last to reappear.

7. Hemocytoblasts, or primordial cells, readily disappeared from the blood. Myeloblasts and promyelocytes, next to disappear, acted in a similar manner, confirming their close morphologic relationship. Myelocytes and metamyelocytes disappeared very slowly, acting more like mature cells. Prolymphocytes and lymphoblasts, usually considered as immature forms of lymphocytes, acted more or less like mature cells under radium.

8. Evidence has not been found of circulating lymphocytes being more affected by radium than the granulocytes. In fact, like the majority of investigators, we have found that, while extensive radiation reduces the absolute number of both types of cells, the relative percentage of lymphocytes is usually increased.

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STUDIES IN BLOOD CELL MORPHOLOGY AND FUNCTION*

No. III—Extreme Neutrophilic Leucocytosis with a Note on a Simplified Arneth Count

By Carlos Pons, M.D., and E. B. Krumbhaar, M.D., Ph.D., Philadelphia, Pa.

EXTREMELY high leucocyte counts (over 60 or 80,000 per c.mm.) are almost always found in clinical medicine to be due to some disease of the blood-making organs, such as leucemia. When rarely found to be due to other causes, they are at least accompanied by the appearance in the peripheral blood of a considerable number of immature forms, such as myelocytes and lymphoblasts, so that a more or less accurate "leucemoid" picture is produced. In neutrophilic leucocytosis, on the other hand, as is well known, the white cell count rarely exceeds 40 to 50,000. Such blood pictures are found in a variety of conditions, such as the various acute infectious, toxic conditions, tumor and after severe hemorrhage. It is perhaps not so well recognized that in these conditions, the proportion of young neutrophiles is particularly increased, and that the combination of hemorrhage and ulcerating cancer are especially potent factors in producing such a leucocytosis, and finally that these very high counts may be produced by a summation of stimuli more easily than by a single factor.

The following case is reported as an example of neutrophilic leucocytosis, greater in magnitude than we have been able to find in the literature, and due to an ulcerated, bleeding cancer, with hemorrhage as the chief factor.

Case: C. S., American, white, female, aged sixty-one, was admitted to the service of Drs. Carnett and Jeffries at the Philadelphia General Hospital on July 8, 1923, with an ulcerated, bleeding breast.

The present illness began two years before, following an injury to the left breast. Three weeks later a lump appeared under the nipple, which gradually grew larger. The left breast had been removed for carcinoma at the Lankenau Hospital, December, 1922. A second operation was performed for recurrence at the same hospital, and since then the patient has felt worse, complaining of cough, loss of weight, and hemorrhage from the breast.

Table I

Blood Findings

DATE	HB.	R.B.C.	W.B.C.	POLYS*	LYMPHO.	MONO.	META-MYELOCYTES	MYELOCYTES
6-11-23	46		44,000	95	4	1	0	0
6-12-23			94,500					
6-13-23			105,000	96	4	0	0	0
6-14-23			106,000	92	2	2	2	2
6-16-23	35	186,000	120,000	92	2	2	0	4

*Unsegmented (young) forms of polymorphonuclear nucleus constantly averaged 82 to 84 per cent of the total leucocyte count.

*From the Laboratory of Clinical Pathology of the Philadelphia General Hospital.
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When admitted to this hospital, she complained of weakness, a craving for water and bleeding from the ulcer in the left breast. When the breast was dressed, the hemorrhages were so profuse as to be alarming. Although there was superficial infection of the ulcer, no signs of sepsis were present. The patient died nine days after admission.

Autopsy.—(Dr. John Eiman, P.G.H. No. 7809). An adult female, weighing 146 pounds. Nutrition very good. The main finding was a large ulcerated cancerous area, beginning at the cartilage of the fifth rib, on the left side, and extending posteriorly to the midline and superiorly from the second rib to the seventh rib anteriorly. The ulcerated area was covered with soft fungous, reddish-yellow tumor masses. Axillary and subclavicular lymphnodes were not enlarged. The left lung showed a metastatic nodule, 4 cm. in diameter. Bone marrow removed from right tibia was dark brick red in color. The other findings were without significance.

Histologic examination confirmed the gross diagnosis of adenocarcinoma and showed an extreme hyperplasia of the bone marrow. Blood culture at autopsy showed B. coli communior.

EXPERIMENTAL WORK

The metastatic nodule in the lung was macerated and injected into rabbits, to try to ascertain whether the tumor had any stimulating properties on the bone marrow with the production of leucocytosis. The results of these injections were entirely negative.

DISCUSSION

In considering the probable factors instrumental in the production of this extreme leucocytosis, the breast cancer was not thought to be solely responsible. The infection also was not considered in itself sufficient to account for the leucocytosis, as it was superficial and accompanied by a negative antemortem blood culture and without constitutional signs of sepsis. Leucocytosis was present several days before the patient was in a moribund condition, so that although this might have affected the last count, it is not in itself sufficient to say that the condition was due to agonal leucocytosis.

The hemorrhages were recurrent and increasingly severe, and it can therefore be inferred that under the stimulating preparation given by the other factors mentioned, the hemopoietic system was able to respond with unusual efficiency to the extra well recognized stimulus of the blood loss.

In regard to the percentages of segmented and nonsegmented nuclei of the polymorphonuclear leucocytes, it has now been our custom for some months to use this subdivision as a simplification of the Arneth[1] formula. Although we believe that this latter represents a true indication of the age of the leucocyte, nevertheless, like many others, we have found it impractical for routine clinical purposes. In the same way the Schilling[2] modification, though a step in the right direction, has also been found too complicated for routine use. We believe that the essential clinical purpose is served if all neutrophiles are subdivided into: 1, metamyelocytes (very young)—with round or slightly indented nuclei; 2, nonsegmented forms (young)—where the nuclear material is connected by broad bands; 3, segmented forms (older)—where two or more groups of nuclear material are joined by narrow filaments. As metamyelocytes, like their immediate ancestors, the myelocytes, are usually segregated in a differential count from the riper cells of the granulocyte

series, this only necessitates the formation of one extra group for routine work. And, as Arneth, Schilling and various other authors have pointed out, information of considerable clinical value can thereby be ascertained, especially in the acute infections. It will be noted that in the present case the number of polymorphonuclear neutrophiles with unsegmented nuclei,

Fig. 1.—Various forms of the neutrophile nucleus, as seen in stained spreads. In the first row are metamyelocytes. In the second row, unsegmented (young) forms. In the third row, segmented (old) forms, with 2 to 6 lobes. The five swellings on the last nucleus of the second row are numbered to show a possible confusion with segmented forms. On account of the thick connecting bands, this is considered an unsegmented young form.

varied from 82 to 84 per cent of the total count, as against the normal of from 4 to 10 per cent. A shift in this direction, though not to such an extreme degree, has regularly been our experience in cases where the polymorphonucleosis is of recent occurrence. It is taken to indicate the response of the leucopoietic tissues to the demand for more leucocytes, whereby relatively younger cells emerge in the peripheral circulation. Such a very high per-

centage of unsegmented forms is, of course, almost as unusual as is the total count itself, the usual increase of these forms in the ordinary leucocytosis being to about 30 or 40 per cent.

SUMMARY

1. A case of extreme leucocytosis occurring in an infected, bleeding carcinoma of the breast is reported (120,000 leucocytes per c.mm.).

2. Several factors were held to be responsible for the leucocytosis: (a) The cancerous growth; (b) Superficial infection; (c) Antemortem leucocytosis; and especially (d) Hemorrhage.

3. A simplification of the Arneth scale is recommended for routine use, as an indication of the age of the leucocyte.

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STUDIES IN BLOOD CELL MORPHOLOGY AND FUNCTION*

IV. The Arneth Count with Supravital Staining under "Living Blood" Conditions

By C. N. Colbert, M.D., Philadelphia, Pa.

THE recent observations of Sabin[1] on supravital staining of preparations of human blood have suggested an additional field for investigation of the behavior of the leucocytes. The problem of the so-called young neutrophile has been an unsettled one, and it was thought that by the examination of supravitally stained preparations it might be ascertained (1) whether the unsegmented nucleus of the young neutrophile ever becomes segmented during examination; (2) whether this change is reversible; and (3) whether the percentage of unsegmented forms is the same in fixed preparations as in the living ones. These findings would have considerable bearing on the value of the Arneth count or its various modifications. No attempt was made to discriminate between forms having different numbers of nuclear segments, and the division into segmented and unsegmented forms was made on the basis suggested in paper III of this series.

METHODS

The technic as described by Dr. Sabin was used in making preparations. Various vital stains were used at first, but it was found that Grubler's cresyl-blue outlined the nucleus most distinctly, and was therefore adopted as routine. It was found that the stain worked best when the saturated alco-

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holic solution was prepared on the same day that the diluted stain was to be used. The life of a preparation in our hands is about three hours, after which time parts of the film become unfit for examination. Janus green, even in small amounts, was too toxic for use.

Blood was obtained from a variety of sources, normal and pathologic. For obtaining large numbers of neutrophiles with a high percentage of unsegmented nuclei, the bloods of a few pneumonia cases showing a high leucocytosis were used. As very thin preparations were necessary definitely to outline the nucleus, the viscosity made considerable difference in the percentage of satisfactory films obtained, and the easiest blood to handle was that of cases of rather marked anemia.

In each preparation, several neutrophiles with unsegmented nuclei were identified, and the outline of each nucleus was repeatedly traced during the life of the film. In no instance did any nucleus, once identified as an unsegmented type, become segmented under observation. No difference in motility or in the nature of the cytoplasm was noted in the two forms.

It was often the case that for some minutes the nature of the nucleus would be in doubt, because of the temporary grouping together of segments and the shortening of the filamentous bands connecting them. When these doubtful nuclei became extended in renewed motion, however, the refractile nuclear segments immediately became distinct. When once these segments were determined in any nucleus, they could be recognized throughout any temporary morphologic changes, and when relieved from pressure, retained their original shape with remarkable constancy. Such a nucleus could not be confused with the unsegmented type.

TABLE I

CLIN. DIAG.	CASE	STAIN	TOTAL LCT. COUNT.	DIFFERENTIAL COUNT IN PERCENTAGES SEGMENTED	UNSEG.	EOSINO.	BASO.	LYMPHO	LARGE & TRANS.
Normal	I	Wright's	9,650	56	1	2	1	37	3
		Vital		55	5	2	0	37	1
Lobar Pneumonia	II	Vital	36,300	46	40	1	0	13	0
				41	44	2	0.5	11	1.5
Lobar Pneumonia	III	Vital	10,200	59	12	1	0	25	3
				53	20	0	1	23	3
Lobar Pneumonia	IV	Vital	24,000	80	6	1.5	1	9.5	2
				79	11	1	1	7	1
Sec. Anemia	V	Vital	5,600	71	3	4	0	21	1
				68	2	3	0	26	1

Differential counts were made on several specimens, grouping all segmented forms together, with the unsegmented forms as a separate class. A check was made by a similar count on a fixed film stained with Wright's stain. Five of these counts are shown in Table I.

In a somewhat greater proportion than in these tables, it was found that the fixed films showed a slightly larger percentage of unsegmented forms than did the vital stain. Some of this difference may be due to the fact that a greater percentage of the unsegmented forms had to be rejected in the

count as not definitely capable of being classified. Undoubtedly some of the horseshoe forms stained with Wright's were actually segmented nuclei in this configuration. Nevertheless the discrepancy is not wide, and the percentage of unsegmented forms is undoubtedly as constant as the percentage of any of the other formed elements of the blood.

COMMENT

Vitally stained living blood films offer an interesting means of corroborating Arneth counts made on stained fixed smears. As the normal life of a leucocyte in the circulation is thought to be only a matter of 2 or 3 days, the constant shape of the neutrophile nucleus in the living state for several hours is further support of the view that this shape is not an accident, but bears a definite relation to the life history of the cell.

A drawback to this method, as Carrel[2] has suggested, is that because of lack of oxygen and fresh media, to remove waste products, the changes are mostly degenerative. The technic could doubtless be modified with this in mind. There are some difficulties in the way of using this method routinely. The percentage of successful preparations is not so great as by polychrome staining. In examining motile neutrophiles, several minutes may be required to determine the nature of each nucleus, making the procedure very lengthy.

SUMMARY

1. Segmentation of the unsegmented neutrophile nucleus was not observed over a period of several hours in preparations of living leucocytes vitally stained.

2. Segmented neutrophile nuclei were not seen to become horseshoe nuclei, although the segments may become temporarily indistinguishable during motion.

3. The relative percentage of segmented and unsegmented nuclei of neutrophiles is approximately the same in living as in fixed preparations.

4. These findings support Arneth's view of the significant relation between the shape of the nucleus and the age of the cell.

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THE USE OF DESICCATED RED BONE MARROW AND SPLEEN IN THE TREATMENT OF ANEMIA*

By William Thalhimer, M.D., Milwaukee, Wis.

Leake[1] and his collaborators, at the University of Wisconsin, have recently reported the results of their investigations of the hematopoietic effect of desiccated red bone marrow and spleen, combined in equal proportions by weight (referred to hereafter as "spleen-marrow compound"), in animals, normal human beings, and patients with anemia. A brief summary of their results is as follows. This preparation when administered either intravenously or orally to rabbits, orally to dogs, and orally to normal beings, caused a very rapid and marked increase in the number of circulating erythrocytes, and a somewhat less increase in hemoglobin. Administration of this preparation by mouth to patients with secondary anemia was followed by prompt beneficial results with a lasting relief of the anemia in most patients. Patients with primary anemia were not benefited, but on the contrary this compound caused a rapid fall in hemoglobin and circulating erythrocytes. This therapeutic test in doubtful pernicious anemia cases can be used as a diagnostic aid.

These investigators have suggested that independent clinical studies be made of the value of spleen-marrow compound, and the following report contains the results of my observations of the effect of this agent in patients with secondary and primary anemia.†

A review of the literature can be found in Leake's articles and need not be repeated here.

METHODS, CLINICAL MATERIAL AND RESULTS

All patients who were given capsules of spleen-marrow compound were carefully followed with erythrocyte counts and hemoglobin determinations. The blood counts were made by experienced technicians, using the usual technic, and either the Dare or Sahli hemoglobinometer. Each patient was followed by the same technician, and the technicians were instructed to be careful not to read the hemoglobin determinations too high. The results can be considered as demonstrating the relative increase and decrease in hemoglobin, even though the clinical hemoglobinometers used might not be relied on for absolute hemoglobin values. The erythrocyte counts are to be relied on both for their absolute and relative values.

The reason several technicians furnished the clinical laboratory work

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†Spleen-marrow compound, already prepared in 5-grain capsules, was secured through the courtesy of Dr. David Klein, of the Wilson Laboratories, Chicago.

is that the observations were made on patients in several hospitals* and on private patients of a number of physicians. I believe that because the patients were followed in several laboratories, the results are more dependable than if the blood counting had been done in a single laboratory. The results of the different technicians automatically act as checks, and actual checks were obtained on several outside patients from time to time by the technician at Columbia Hospital, the blood counts agreeing within the usual limits of error.

Practically all the patients were given three 5-grain (0.3 gram) capsules of the preparation a day; i.e., one capsule before or after each meal. No side actions were noted. No selection of patients was made.

The table of the results is practically self-explanatory and needs very little amplification. The records are reported as they stand at this time. Some patients have been followed long enough to give final results. Others have been followed only for a short time. The results are arranged in no special order except that grouping has been made of patients with secondary anemia showing improvement, those showing no improvement, and those with pernicious anemia.

Forty-six patients with secondary anemia have received spleen-marrow compound. Forty-one patients (89 per cent) showed an increase in circulating erythrocytes and hemoglobin, and five patients (11 per cent) did not. In many patients an increase of the erythrocytes occurred in from one to three days. The increase in the hemoglobin was usually more tardy and its continued rise more gradual. Some of the patients showed an increase in hemoglobin from an initial value of 60 per cent to a final value of about 90 per cent, and of erythrocytes from about 3,000,000 at the beginning of treatment to about 5,000,000 per cubic millimeter in several weeks.

Two patients (16 and 20) had been given iron and arsenic intravenously with slight or no improvement. Spleen-marrow compound was then given and improvement followed.

One patient (8) who improved after a short term of medication, but before a lasting effect was produced, refused to continue treatment. The blood count and hemoglobin promptly fell.

In a few anemic patients (5, 7, 25) whose blood findings rose very rapidly to normal, or slightly above normal, medication was immediately discontinued. A week later a moderate fall in the blood count was found. Treatment was resumed and was followed by a prompt rise in erythrocytes and hemoglobin. This evidence indicates a direct relationship between administering the extract of red bone marrow and spleen and the increase in the patient's hemoglobin and circulating erythrocytes.

Although in some patients the blood findings increase markedly or even return to normal after only two or three weeks, it seems that treatment must be continued for six or eight weeks for the improvement to become permanent.

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*These clinical observations were made possible only through the cooperation of a number of physicians. I am particularly indebted for this cooperation to Doctors C. H. Stoddard, A. J. Patek, David Fisher, (of the Staff of the National Soldiers' Home), Francis McMahon, A. B. Schwartz, W. T. Nichols, R. W. Roethke, G. A. Carhart, W. L. LeCron and J. L. Yates.

TABLE OF RESULTS

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
1	Mitral Stenosis and Regurgitation. Male, age 51.	2-9	2-9	3,790,000	70	
			2-10	3,920,000	75	
			2-11	4,035,000	80	
			2-18	4,410,000	88	
			2-25	4,060,000	87	Patient feels much improved, less dyspnea.
2	Postoperative suppuration of wounds. Male, age 26.	2-9	2-9	3,170,000	60	
			2-10	3,750,000	70	
			2-11	4,360,000	78	
			2-13	4,235,000	82	
			2-18	4,280,000	80	
3	Postoperative suppuration of wounds. Male, age 32.	2-9	2-9	2,720,000	50	
			2-10	3,300,000	58	
			2-11	3,220,000	61	
			2-13	3,290,000	63	
			2-19	3,570,000	70	
			2-25	3,480,000	75	
			3-3	3,160,000	75	
4	Chronic Pleurisy with effusion. Male, age 21.	2-9	2-9	2,880,000	50	
			2-10	3,220,000	55	
			2-11	3,220,000	60	
			2-13	3,710,000	72	
			2-18	3,820,000	71	
			2-25	4,810,000	88	
5	Menorrhagia. Age 39.	2-10	2-10	3,160,000	60	
			2-13	4,080,000	72	
			2-15	5,580,000	88	Medication discontinued.
			2-20	4,720,000	79	Medication resumed.
			2-25	5,200,000	91	
6	Lung abscess	2-12	2-12	3,780,000	65	
			2-13	3,232,000	66	
			2-18	3,400,000	65	
			2-20	4,080,000	70	
			2-22	4,101,000	70	Medication discontinued.
			3-10			Medication resumed.
			3-18	4,180,000	76	
			3-21	5,320,000	84	
7	Bilateral Pyonephrosis. Male, age 33.	2-13	2-13	3,490,000	63	
			2-14	3,070,000	60	
			2-15	3,310,000	64	
			2-18	4,210,000	84	Medication discontinued.
			2-25	3,720,000	68	Medication resumed.
			2-29	4,320,000	84	

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
8	Diagnosis undetermined. Male, age 68.	2-15	2-15	3,550,000	70	
			2-18	4,410,000	86	Patient refused to take capsules after this.
			2-25	3,610,000	68	
			3-3	2,860,000	64	
9	Suppurating tuberculous hip. Male, age 25.	2-16	2-16	3,540,000	62	
			2-17	3,660,000	57	
			2-20	3,385,000	65	Patient refused to take capsules because he was suffering from abdominal cramps.
10	Polyarticular Arthritis. Male, age 29.	2-20	2-20	3,530,000	70	
			2-22	3,480,000	74	
			2-27	4,100,000	80	
			3-3	4,390,000	86	
11	Hemolytic Icterus splenectomy, 11 mos. ago. Female, age 24.	1-22	1-22	3,580,000	71	
			1-29	3,580,000	76	
			2-13	3,750,000	73	
			2-18	3,910,000	75	
			2-26	4,110,000	79	
			3-11	4,160,000	78	
12	Secondary anemia, cause not discovered. Female, age 18.	2-14	2-14	3,260,000	49	
			3-5	3,520,000	53	
			3-14	4,010,000	58	
13	Secondary anemia, cause not discovered. Female, age 27.	2-4	2-4	3,700,000	79	
			2-23	4,340,000	83	
			3-2	4,320,000	84	
14	Secondary anemia, cause not discovered. Female, age 32.	1-30	1-30	4,250,000	81	
			2-7	4,400,000	83	
			2-13	3,920,000	83	
			2-20	4,290,000	87	
15	Secondary anemia, cause not discovered. Female, age 36.	1-8	1-8	4,070,000	76	
			1-15	4,310,000	76	
			1-23	4,020,000	80	
			1-30	4,010,000	77	
			2-13	4,340,000	80	
			2-20	4,200,000	85	
			3-5	4,350,000	84	
			3-14	4,380,000	86	
16	Secondary anemia, cause not discovered. Female, age 22.	1-1	1-1	4,000,000	57	Previous to taking this preparation the patient had three intravenous injections of iron and arsenic with no relief of the anemia.
			1-5	4,270,000	64	
			1-12	4,440,000	69	
			1-19	4,280,000	70	
			1-27	4,280,000	70	
			2-2	4,440,000	76	

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
17	Secondary anemia, cause not discovered. Female, age 25.	2-18	2-18	3,110,000	77	
			2-28	3,850,000	78	
			3-6	4,070,000	82	
18	Secondary anemia, cause not discovered. Female, age 30.	1-28	1-28	3,520,000	74	
			2-8	3,760,000	79	
19	Secondary anemia, cause not discovered. Female, age 38	11-22-23	11-22	3,230,000	60	
			12-6	4,360,000	71	
			12-13	4,990,000	83	
			1-5	5,030,000	90	
20	Secondary anemia, infantile uterus, menorrhagia, menstrual blood loss less than normal.	11-19-23	11-19	4,010,000	84	Oct. 15, 1923 the hemoglobin was 73% and after two intravenous injections weekly of iron and arsenic for five weeks the hemoglobin on Nov. 19, 1923, was 84%.
			12-3	4,670,000	96	
21	Secondary anemia menorrhagia, age 27.	11-21-23	11-21	4,080,000	76	
			12-6	4,010,000	84	
			1-8	4,450,000	88	
22	Secondary anemia cause not discovered. Female, age 44.	10-19-23	10-19	4,030,000	43	Shape of erythrocytes suggestive of pernicious anemia.
			11-16	4,200,000	51	
23	Secondary anemia Pulmonary tuberculosis suspect. Female age 24.	11-22-23	11-22	4,030,000	78	
			12-10	4,430,000	84	
			1-27	4,650,000	91	
24	Secondary anemia cause not discovered. Female, age 18.	2-23	2-23	4,640,000	70	
			3-5	4,040,000	69	
			3-11	4,940,000	71	
25	Ruptured Ectopic pregnancy. Age 33.	12-18-23	12-3	3,990,000	41	Operation Dec. 10. Diagnosis verified.
			12-18	3,630,000	50	Right tube and ovary removed and as much as possible of intraperitoneal blood clot was removed.

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
25 Cont'd			12-20	4,200,000	67	
			12-22	4,530,000	68	
			12-24	4,370,000	68	
			12-26	4,000,000	71	Patient went home but continued medication.
			1-15	4,720,000	80	
			2-15	5,710,000	95	Discontinued medication.
			3-4	4,580,000	83	Resumed medication.
26	Secondary anemia, acute otitis media. Female, age 40.	2-7	2-7	4,260,000	78	
			2-18	4,250,000	79	
			3-4	4,650,000	82	
27	Slight anemia, patient felt weak and looked paler than her blood count would indicate. Female, age 23.	2-8	2-8	4,540,000	90	
			2-15	5,100,000	95	Medication discontinued.
28	Secondary anemia, cause not discovered.	2-15	2-15	3,450,000		
			2-21	3,720,000	75	
			2-26	3,870,000	80	
			2-28	3,920,000	80	
			3-11	3,920,000	78	
29	Secondary anemia, cause not discovered. Male, age 28.	3-1	3-1	3,500,000	68	
			3-10	4,720,000	100	
30	Active pulmonary tuberculosis. Male, age 28.	2-23	2-23	4,800,000	90	
			2-25	5,200,000	90	
31	Active pulmonary tuberculosis. Male, age 30.	2-23	2-23	4,400,000	85	
			2-27	4,920,000	91	Medication discontinued.
			3-12	4,290,000	78	Medication resumed.
			3-18	4,830,000	88	
			3-21	4,288,000	89	
			3-25	4,520,000	87	
32	Active pulmonary tuberculosis. Male, age 26.	2-23	2-23	4,288,000	85	
			2-27	4,736,000	88	
33	Active pulmonary tuberculosis. Male, age 27.	2-23	2-23	4,168,000	90	
			2-26	5,180,000	100	
34	Postoperative infection, (cesarean section.) Jan. 5. Age, 33.	2-7	1-12	4,850,000	88	
			1-18	3,910,000	60	
			2-7	3,480,000	53	
			2-11	3,900,000	51	
			2-13	3,900,000	55	Patient left hospital, no further observations.

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
35	Active pulmonary tuberculosis. Male, age 34.		3-3	4,190,000	90	
			3-12	4,830,000	94	
			3-18	5,090,000	95	
			3-21	5,160,000	95	
			3-25	5,000,000	96	
36	Secondary anemia, cause not discovered. Female, age 30.	3-14	3-14	3,590,000	60	
			3-15	4,040,000	60	
			3-16	4,320,000	62	
			3-17	4,360,000	65	
37	Postoperative rectal hemorrhage, (Oper. for prolapse of rectum). Male, age 29.	3-20	3-20	2,340,000	45	
			3-23	3,010,000	60	
38	Fibromyoma of uterus and bleeding hemorrhoids. Age 38.	3-12	3-12	3,980,000	75	
			3-15	4,590,000	80	Medication discontinued. Hysterectomy, performed Mar. 18.
			3-21	3,560,000	64	Medication resumed.
			3-23	4,080,000	77	
39	Von Jaksch anemia. Male, age 2.	3-16	3-14	1,600,000	45	W. B. C. 25,000 no abnormal leucocytes, but 7 normoblasts per 100 leucocytes.
			3-17	1,570,000	47	W. B. C. 15,000; 4 normoblasts.
			3-18	2,000,000	48	W. B. C. 13,000; 3 normoblasts.
			3-19	2,060,000	48	W. B. C. 10,200; no normoblasts.
			3-20	2,060,000	53	W. B. C. 10,000; no normoblasts.
			3-21	2,640,000	53	W. B. C. 10,400; no normoblasts.
			3-22	2,500,000	54	W. B. C. 10,000; no normoblasts.
			3-23	2,630,000	54	W. B. C. 10,200; no normoblasts.
			3-24	2,860,000	60	W. B. C. 11.000; no normoblasts.
			3-25	2,900,000	60	W. B. C. 10,000; no normoblasts.
			4-1	3,900,000	70	
40	Mild purpura-occasional small skin hemorrhages. Female, age 34.	2-15	2-19	4,000,000	75	
			2-29		80	
			3-13	4,300,000	83	
41	Secondary anemia, cause not discovered. Female, age 22.	12-10-23	12-10	3,900,000	78	
			12-21	4,130,000	82	
			12-29	4,150,000	86	
			2-1	4,000,000	85	Menstruating at present.

Patients with Secondary Anemia Showing no Improvement

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
42	Multiple abdominal sinuses after multiple intra-abdominal abscesses following acute appendicitis.	11-5-23	11-5	4,740,000	82	
			11-8	4,650,000	85	
			11-16	5,000,000	85	
			11-26	5,250,000	86	
			12-1	4,750,000	84	
			12-8	4,690,000	82	
			12-18	4,750,000	84	
43	Six months' pregnancy. No reason found for anemia. Age, 24.	2-8	2-8	3,970,000	63	
			2-27	3,600,000	57	
			3-5	3,630,000	42	Medication stopped.
44	Child, male, age 3 yrs., history of intestinal worms. Stool examination negative.	2-19	2-19	3,920,000	52	
			2-26	3,950,000	53	
			3-10	3,780,000	42	
45	Secondary anemia, Female, age 70 yrs. Malignancy of stomach suspected.	2-26	2-26	4,560,000	88	
			3-5	4,500,000	86	
			3-11	4,460,000	82	
46	Active pulmonary tuberculosis.	2-24	2-24	4,288,000	85	
			2-26	4,160,000	88	
			2-27	4,730,000	88	
			3-12	4,560,000	84	
			3-18	4,290,000	86	
			3-21	4,180,000	87	
			3-24	4,110,000	86	

Patients with Pernicious Anemia

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
47	Pernicious Anemia		1-22	2,500,000	62	
		1-23	1-25	1,250,000	45	Medication discontinued.
			2-19	1,800,000	58	
48	Pernicious Anemia	2-16	2-16	2,020,000	65	Diagnosis not clear before medication.
			2-19	1,200,000	43	Medication discontinued.
49	Pernicious Anemia	2-18	2-18	1,400,000	30	
			2-19	1,232,000	29	
			2-20	992,000	30	Medication discontinued.
50	Pernicious Anemia	1-2	1-2	3,610,000	67	
			1-5	2,980,000	60	
			1-7	2,520,000	52	Medication discontinued.

In four patients with pernicious anemia, administration of the capsules caused a prompt decrease in both hemoglobin and circulating erythrocytes. Therefore, this preparation should not be used in this condition. However, one patient with Von Jaksch's anemia (39), has shown a marked improvement in both clinical condition and blood picture during ten days of treatment. The red cell count increased from 1,600,000 to 2,900,000 per cubic millimeter, and the hemoglobin from 45 per cent to 60 per cent. The leucocyte count decreased from 25,000 to 10,000 per cubic millimeter, and normoblasts which were present before treatment in the ratio of seven to every one hundred leucocytes, have not been present since the third day after spleen and bone-marrow medication was begun.

It is interesting that in one patient with hemolytic icterus, whose spleen had been removed eleven months previously, hematopoiesis was stimulated by the preparation.

The satisfactory response of the patients with pulmonary tuberculosis is also of interest, and may be of considerable importance.

In all patients, subjective improvement accompanied the relief of the anemia. It must be remembered that vigilance should not be relaxed in attempting to determine the cause of the anemia, even though improvement follows red bone marrow and spleen therapy in some cases of secondary anemia which have an obscure etiology.

SUMMARY

The administration of desiccated spleen and red bone marrow, combined in equal proportions, in three five-grain capsules daily, to a group of forty-six patients with secondary anemia was followed by a definite increase in hemoglobin and circulating erythrocytes in 89 per cent of these patients.

This preparation caused a fall in hemoglobin and circulating erythrocytes in four patients with pernicious anemia, and therefore, should not be used in this condition.

These results are confirmatory of the observations of Leake and his collaborators.

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THE REMOVAL OF PHENOLTETRACHLORPHTHALEIN FROM THE CIRCULATION*

By R. Ottenberg, M.D., S. Rosen, M.D., and L. Goldsmith, M.D.,
New York City

PHENOLTETRACHLORPHTHALEIN, when injected into the blood, is excreted by the liver. Rosenthal's method of testing liver function consists of injecting the dye and testing the blood after fifteen and sixty minutes to see how much of the dye has been removed. We have used this method in more than 100 cases.

Some interesting problems are brought up by observations on the percentage of dye in the blood during the first fifteen minutes after intravenous injection. In fourteen cases a needle was inserted in a vein of the opposite arm and samples of blood were obtained at intervals varying from one, two, three, five or ten minutes after completion of the injection. The data are given in the accompanying chart. In all these cases the amount of dye injected was 5 mg. per kilo of body weight.

The most interesting point is the great speed with which initial removal of the dye from the circulation takes place. Whether there was ultimately dye retention or not, the percentage of dye was reduced from the theoretical 100 per cent which would have been present if all injected were in the circulation, to 20 per cent or less, within the first five minutes. In fact in all the cases in which a two or three minute observation was made it was found that three-fourths of the dye had already been removed from the blood.

Of course, some irregularities are due to the fact that it probably takes several minutes for the dye to become uniformly distributed in the circulation. Also it must be remembered that removal of the dye begins at the beginning of the injection, and the giving of the injection consumes from one to five minutes.

We can understand the rapid removal of the dye when we remember that in volume of blood supply the liver stands first of all the organs of the body. In a dog of 14,300 gm. weight, with a liver weighing 454 gm., Burton Opitz† found that the liver received 422 c.c. of blood per minute, or roughly half the blood in the body. At this rate, assuming perfect mixing, 75 per cent of the body blood should pass through the liver in two minutes and 96 per cent in five minutes.

This initial rapid removal of dye takes place, whether the liver is able to excrete dye or not. In the chart it will be seen that (with the exception of one case in which no reading was made between three and fifteen minutes) the initial drop occurred in all the cases in which the one hour blood showed

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†Quart. Jour. Exper. Physiol., 1911, iv, 116.

10 per cent or more of dye, i.e., cases in which the liver was unable to excrete the dye. The initial removal therefore does not depend on excretion by the liver, but on absorption from the blood. The percentage of dye in the circulation after fifteen minutes represents an equilibrium between the pressure of dye in the blood and the absorbing capacity of the tissues. This point of balance is, of course, lower in the instances in which the liver has already been able to excrete some dye than in those in which the liver is obstructed. The mechanism of this removal requires study; at present it cannot even be stated whether it is by the reticuloendothelial (Kupfer) cells or not, and if by reticuloendothelial cells whether exclusively by those of the liver or also by others.

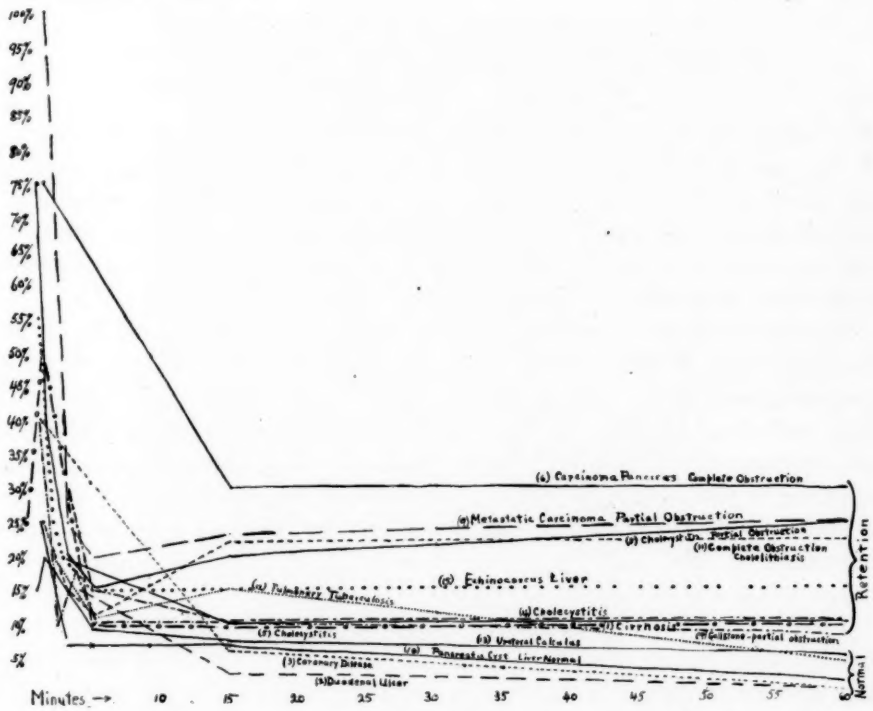

Chart 1.

This offers a partial explanation of the fact that in cases in which the liver is completely obstructed or is badly damaged the percentage of dye in the blood is usually the same at one hour as at fifteen minutes (twenty-three of our 102 miscellaneous cases). It does not, however, explain those cases (nine in number) in which the one hour concentration of dye was higher than the fifteen minute. When we first encountered this, we thought it might be an error, but subsequent observations convinced us it was a reality. We can offer no satisfactory explanation of it, though possibly it may be due to failure of the dye to become uniformly distributed throughout the circulation even in fifteen minutes.

That the body has resources for handling the dye other than excretion by the liver is evident from: (1) the fact that nobody has been able to

account for excretion of more than 75 per cent of the injected dye; (2) in cases of total biliary obstruction the dye disappears from the circulation in twenty-four to forty-eight hours. One of these resources is excretion by the kidneys. This occurs, as shown by Whipple, Peigtal and Clark, only when the liver is badly damaged. We can corroborate this from experience with clinical cases. It is doubtful, however, whether the amount excreted by the kidneys can account for more than a small part of the dye injected.

LESIONS IN GUINEA PIGS PRODUCED BY INOCULATION OF VERY OLD CULTURE OF TUBERCLE BACILLUS*

By Randle C. Rosenberger, M.D., Philadelphia, Pa.

MY reason for making the following observations upon an old culture of the human tubercle bacillus, was to see whether it would eventually bring about death of an inoculated animal. In previous experiments, the period of observations had been only six to twelve weeks. With small doses of culture only very small lesions would devleop, and often no perceptible lesions were observed at autopsy.

The culture used in the experiments was originally isolated by Trudeau at Saranac Lake, in 1891, directly from a child's lung and called "R_1" bacillus tuberculosis hom. Baldwin has stated: "It has passed through one rabbit, and since grown on glycerin sheep serum, glycerin agar and glycerin broth successively. During the last ten years it has been grown upon broth containing 5 per cent glycerin and reaching slightly acid; a new transfer was made nearly every month. Its virulence for rabbits and guinea pigs was pronounced at first, but the present culture barely infects guinea pigs even in large doses and then only very slight localized or chronic disease results."

I received this culture in July, 1904, and since that time have kept it growing upon 6 per cent glycerin agar, making subcultures every two or three months during this interval of twenty years. As the organism was originally isolated in 1891, at the time of this experiment it had been under cultivation for thirty-three years.

Two pigs were inoculated intraperitoneally with 2.5 c.c. of a salt suspension of a glycerin agar growth of the strain "R_1" tubercle bacillus, on November 8, 1921.

May 11, 1922, or six months after inoculation, one pig was sacrificed and no appreciable emaciation was noticed.

Upon opening the abdominal cavity a large hour-glass shaped mass 7.5 cm. in length and 2 cm. in thickness and distinctly caseous was found. In addition, small nodules or enlarged glands were observed varying from 0.5 cm. to 1.5 cm. in diameter beneath the diaphragm and one nodule was in the

*From Jefferson Medical College, Philadelphia.

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diaphragm. One small mass was in the lower border of the right lobe of the liver; one nodule, 1 cm. in diameter, was in the tail of the spleen; one in the epididymis; the left testicle was caseous and a globular mass, 2.5 cm. in diameter, (caseous) was adherent to the posterior wall of the abdominal wall on the right side. No microscopic lesions were present in the lungs or kidneys.

Spreads made from all of these nodules contained tubercle bacilli and a few were found in the urine obtained from a greatly distended bladder.

Inoculations were made upon blood serum and glycerin agar, but no growth occurred up to four weeks' time.

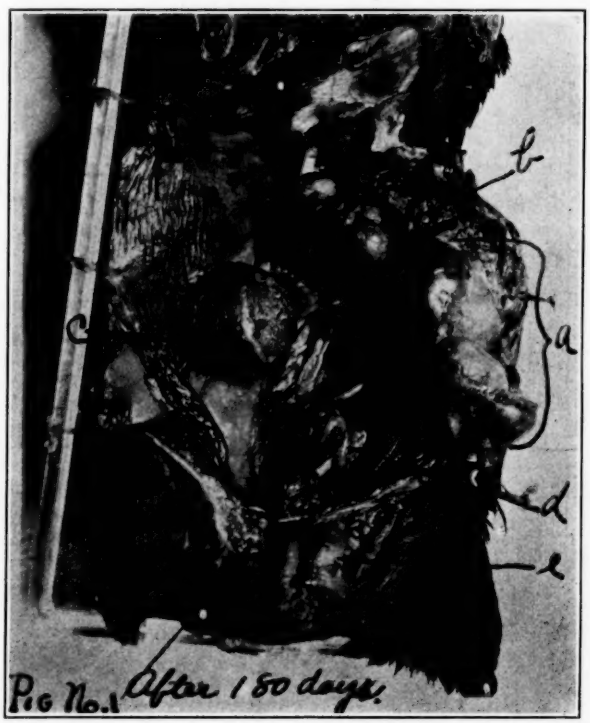

Fig. 1.—Pig No. 1, after 180 days. *A*, Mass in abdomen; *B*, nodule upon tail of spleen; *C*, mass adherent to abdominal wall; *D*, lesion in pelvis anterior to rectum; *E*, caseous testicle.

The second pig was allowed to live and the animal was found dead in its cage October 13, 1923.

During the life of the animal, more especially after it had survived the inoculation for about a year, it was noticed that upon a number of occasions it would sit very quietly, objected to being handled, its hair was ruffled and upon examination a small mass was appreciable by palpation in the abdomen.

It would move about with great deliberation, and its bodily movements were not those of a normal animal. During the last few months of its life it appeared listless, would seek the corner of its cage and remain very quiet.

Emaciation gradually set in and death occurred within slightly less than two years' time.

At autopsy, a large mass almost globular, 6 cm. in diameter was found in the abdominal cavity, distinctly caseous, and with adhesions to the large and small intestines. In addition to this large mass, a smaller one was found (also caseous) between the bladder and rectum, and a caseous lesion was adherent to the diaphragm and superior surface of the liver. Firm adhesions were noticed at the base of both lungs, though no gross lesions were present in these organs.

There were a number of adhesions present at the tail of the spleen but no gross lesions were found in this organ, or in the kidneys or liver.

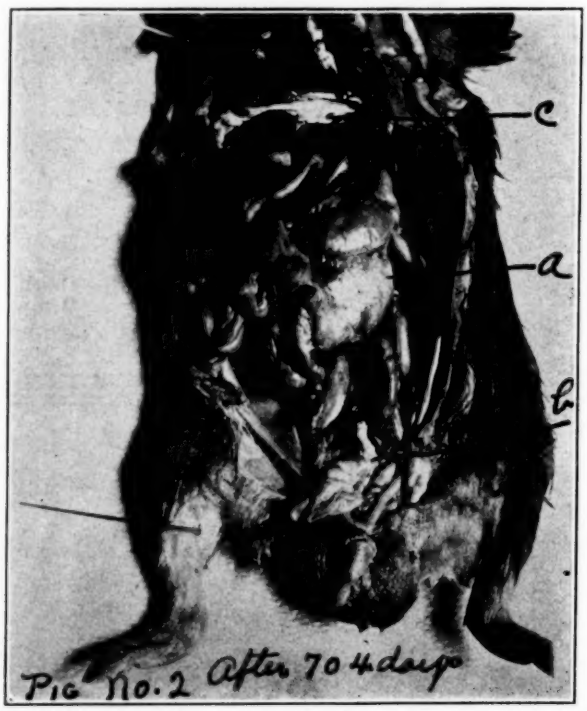

Fig. 2.—Pig No. 2, after 704 days. *A*, Large caseous mass adherent to stomach and intestines; *B*, caseous mass between bladder and rectum; *C*, masses between liver and diaphragm.

Spreads made from all the caseous lesions showed tubercle bacilli. Inoculations were made from the caseous mass in the abdominal cavity into glycerin bouillon but no increase in the number of organisms was observed up to six weeks.

Histologic examination was made of the kidneys, lungs, liver and spleen, but no distinct lesions of tuberculosis were found. The lungs showed a markedly cellular pneumonia, but no tubercles.

This strain of tubercle bacillus, after thirty-three years of cultivation, was capable of producing massive lesions in guinea pigs and ultimately caused the death of the animal studied.

LABORATORY METHODS

A MODIFICATION OF THE BLOOR METHOD FOR BLOOD PHOSPHATES*

By K. Lucille McCluskey, Ph.D., Chicago, Ill.

Briggs'[1] modification of the Bell and Doisy method for the determination of inorganic and acid soluble phosphoric acid in the blood appears to be more desirable than previous ones due to its comparative simplicity and equal accuracy.

For a study on the distribution of phosphorus in the cell and plasma of tuberculous blood, it occurred that possibly an extension of the above method to the determination of total and lecithin phosphorus might be advisable. This has been accomplished by a combination of the Briggs' method with that of Bloors.[2]

It was found that a phosphate content ranging from 0.09 mg. to 0.18 mg. of H_3PO_4 when reduced with hydroquinone in acid sulphite solution gave a color of desirable intensity for measurement in a volume of 15 c.c. to 20 c.c. This amount of phosphate corresponds to that found in the samples used for analysis in the Bloor method. For this reason the quantities used here for the analyses have been the same. The method of Bloor has been followed for the preparation and digestion of the blood solutions and should be consulted for details.

Total phosphorus, lecithin, acid soluble and inorganic phosphorus were determined upon whole blood and plasma. The acid soluble and inorganic phosphorus determinations were made upon filtrates obtained by deproteinizing whole blood in some cases with acid ammonium sulphate and in others with trichloracetic acid.

A detailed account of the technic used, is given for the total phosphates. This procedure was followed exactly in all the determinations with the exception of such modifications as are indicated below.

METHOD

Total Phosphates.—To 3 c.c. of whole blood in a 25 c.c. volumetric flask, water is added to the mark and the solution is well mixed. One c.c. of this solution is measured into a large tube (25 mm. x 250 mm.) graduated at 15 c.c. and digestion is carried out over a microburner after 1.5 c.c. of a mixture of equal parts of concentrated sulphuric and nitric acids and two glass beads have been added. After digestion is complete the solution is cooled, 5 c.c. of water added, and the acid titrated with approximately 40

*From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Illinois.
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per cent NaOH (from sodium), using phenolphthalein as indicator. The solution is brought back to the slightly acid side by adding an approximately N/1 H_2SO_4 drop by drop until the color just disappears.

Standard Phosphate Solutions.—To each of two digestion tubes are added an amount of alkali equal to that used in the neutralization of the digestion mixture above and one drop of phenolphthalein. The solution is made acid with dilute sulphuric acid, then just alkaline with one or two drops of sodium hydroxide and brought to the neutral point with the N/1 H_2SO_4. The tubes are cooled, and to one are added 4 c.c. and to the other 5 c.c. of standard phosphate solution, containing respectively the equivalent of 0.12 mg. and 0.15 mg. of H_3PO_4. Then to both the unknown and the standards are added 2 c.c. of Na_2SO_3, 1 c.c. of hydroquinone and the tubes made up to the 15 c.c. mark with water. After the addition of 2 c.c. of ammonium molybdate solution, the tube is at once stoppered tightly with a good cork stopper, well mixed, allowed to stand two to three hours, and read in the colorimeter.

Colorimeter Reading.—The 5 c.c. standard is placed in the colorimeter on the left and the 4 c.c. standard on the right and set at 30.

The standards are considered to be proportional to their phosphate content, if the average of several readings of the 5 c.c. standard lies between 23.7 and 24.3. The unknown is then matched with the standard having the nearer color intensity. Measurements were made in the Kober colorimeter with the standard set at 30.

NOTES ON METHODS

The standards for lecithin and inorganic phosphorus should contain the equivalent of .09 mg. and .12 mg. of H_3PO_4.

The acid soluble phosphorus determinations require a standard of .15 mg. or .18 mg. of H_3PO_4 for whole blood and .15 mg. of H_3PO_4 for plasma.

The standard for the inorganic phosphorus determination made on the acid ammonium sulphate filtrate is made to contain 8 c.c. of acid ammonium sulphate and a period from fourteen to seventeen hours should be allowed for reduction.

To balance the salt content, 1.5 c.c. of acid ammonium sulphate is added to the standard for the acid soluble phosphorus determination of this same filtrate, and the period of reduction is six hours.

The average acidity found by titrating 10 c.c. portions of the filtrates from the trichloracetic acid precipitation corresponds to .75 c.c. of the trichloracetic acid solution. The standards for the inorganic phosphorus are made to contain this amount.

It was observed that when the tubes were left exposed to the air, during the period of reduction, a deep blue layer formed at the surface and gradually spread throughout the tube. This oxidation is prevented by the presence of an atmosphere of sulphur dioxide. For this reason a large quantity of sodium sulphite solution has been used, and the tubes stoppered tightly during the reduction.

Since the temperature affects the speed of reduction of the phosphomolybdic acid, it is necessary before the reagents are added that a uniform

temperature exist. As a matter of routine, the tubes were cooled under the tap to room temperature after neutralization or were allowed to stand until such temperature was attained. During the neutralization process on account of the heat developed and the strong acid solution, the phenolphthalein was not added until most of the acid had been neutralized and the tube cooled.

It was observed that if the molybdate reagent was added in presence of ammonium sulphate and the tubes allowed to stand as short a time as two minutes before the other reagents were added, a faint yellow color became visible and upon the addition of the sulphite and hydroquinone, a greenish blue color developed immediately which deepened to a dark blue within a short time and was not proportional to the phosphate content. If the reagents are added in the order of sulphite, hydroquinone, and molybdate, this difficulty is eliminated and although the color development is slower in presence of the ammonium salts, it is strictly proportional to the phosphate content. This order of adding reagents has been adopted throughout.

The digestion tubes were graduated at 15 c.c.

Should the tubes become alkaline after the addition of the sodium sulphite, they are made just acid with N/1 H_2SO_4 before the ammonium molybdate solution is added.

To ascertain whether the color intensity was still proportional to the phosphate content within the time limits allowed for reduction, the following experiments were tried.

1. One and five-tenths c.c. of sodium hydroxide were measured into each of two tubes and neutralized with dilute sulphuric acid, 4 c.c. of standard phosphate were added to one tube and 5 c.c. to the other. After the addition of 2 c.c. of Na_2SO_3 1 c.c. of hydroquinone, and 2 c.c. of ammonium molybdate, the tubes were made to the 15 c.c. mark, stoppered, mixed and allowed to stand. The readings were as follows:

AFTER 50 MIN.		AFTER 5 HRS.		AFTER 24 HRS.	
5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.
23.8	30	23.8	30	24.3	30

2. The above experiment was repeated with the addition of 1.5 c.c. of acid ammonium sulphate to each tube. The following readings were taken:

AFTER 75 MIN.		AFTER 3 HRS.		AFTER 23 HRS.	
5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.
22.7	30	24.1	30	25.2	30

3. Eight c.c. of acid ammonium sulphate were measured into each of two tubes. To one were added 3 c.c. and to the other 4 c.c. of standard phosphate followed by 2 c.c. of Na_2SO_3, 1 c.c. of hydroquinone, and 2 c.c. of ammonium molybdate and the volume made to 17 c.c. The readings were as follows:

AFTER 1.5 HRS.		AFTER 18 HRS.		AFTER 46 HRS.	
4 c.c. std.	3 c.c. std.	4 c.c. std.	3 c.c. std.	4 c.c. std.	3 c.c. std.
Color too faint to read		22.1	30	22.4	30

RESULTS AND DISCUSSION

In Tables I and II are reported the results obtained by the described technic upon bloods from patients at the Chicago Municipal Tuberculosis Sanitarium.

TABLE I

PHOSPHORIC ACID COMPOUNDS IN WHOLE BLOOD IN TUBERCULOSIS
MG. OF H_3PO_4 PER 100 C.C.

INITIAL AND CASE NO.	SEX	CORPUSCLE VOLUME	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS $(NH_4)_2$ SO_4 FILTRATE	LECITHIN PLUS ACID SOLUBLE PHOSPHORUS	T-(L PLUS ASP)	INORGANIC PHOSPHORUS	N. T. A. CLASSIFICATION
H. B. 17022	♂	47.2	137.3	39.8	97.9	137.7	−0.4	14.7	Inc. B
P. G. 17058	♂	36.2	132.7	36.1	95.0	131.1	+1.6	14.4	F. A. B.
R. P. 17012	♂	43.4	130.2	40.9	86.7	127.6	+2.6	14.5	Mitral Stenosis Under Observation for TB.

TABLE II

PHOSPHORIC ACID COMPOUNDS IN WHOLE BLOOD IN TUBERCULOSIS
MG. OF H_3PO_4 PER 100 C.C.

INITIAL AND CASE NO.	SEX	CORPUSCLE VOLUME	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS TRICHLORACETIC ACID FILTRATE	LECITHIN PLUS ACID SOLUBLE PHOSPHORUS	T-(L PLUS ASP)	INORGANIC PHOSPHATE TRICHLORACETIC ACID FILTRATE	N.T.A. CLASSIFICATION
E. L. 17037	♀	41.4	115.2	31.0	81.1	112.1	+3.1	11.0	M.A.A.
A. P. 17032	♀	39.4	114.0	29.0	82.0	111.0	+3.0	10.4	M.A.A.
A. L. 17031	♀	39.9	118.5	36.9	82.1	119.0	−0.5	11.4	M.A.B.

TABLE III

INORGANIC PHOSPHORIC ACID MG. PER 100 C.C. WHOLE BLOOD

CASE NO. AND INITIAL		PER CENT OF CELLS	TRICHLORACETIC ACID FILTRATE				AMMONIUM SULPHATE FILTRATE		
			After 45 minutes	After 4 hrs.	After 18 hrs.	After 24 hrs.	After 4 hrs.	After 17 hrs.	After 24 hrs.
H. B.	17012	47.2	12.4	14.6	21.0	21.0	Color too faint to read	14.7	14.7
P. G.	17022	36.2	13.7	15.6	21.8	21.8	Color too faint to read	14.4	14.9
R. P.	17058	43.4	12.2	14.5	20.3	20.0	Color too faint to read	14.5	14.3

There is one disadvantage in determining the inorganic phosphate in an ammonium sulphate filtrate by the above technic, on account of the length of time necessary for color development. It has been pointed out by Bloor, Bell and Doisy that the inorganic fraction increases on standing.

Such an increase was observed here, but it was found to be greater in

TABLE IV

DISTRIBUTION OF PHOSPHORIC ACID COMPOUNDS IN WHOLE BLOOD AND PLASMA
MG. OF H_3PO_4 PER 100 C.C.

INITIALS AND CASE NO.	SEX	CORP. VOLUME	TOTAL PHOSPHORUS		LECITHIN PHOSPHORUS		ACID SOLUBLE PHOSPHORUS		SUM OF L PLUS ASP		T–(L PLUS ASP)		INORGANIC PHOSPHORUS		N. T. A. CLASSIFICATION
			Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	
M. M. 18095	♀	40.4	112	34.4	33.5	22.3	78.3	11.1	111.8	33.4	+0.2	+1.0	13.0	10.8	M. A. B.
G. C. 18093	♀	40.2	110.4	31.6	30.9	21.1	76.7	10.9	107.6	32.0	+2.8	–0.4	11.7	10.4	M. A. B.
A. O. 17092	♂	45.6	120.9	26.9	34.7	17.0	84.5	10.3	119.2	27.3	+1.7	–0.4	10.9	8.8	M. A. B.

the trichloracetic acid filtrate than in the acid ammonium sulphate filtrate. This is indicated by the following experiments:

In one experiment, the trichloracetic acid and the ammonium sulphate precipitations were carried out immediately after the bloods were drawn. The tubes were prepared as usual and the readings made at various intervals. In Table III are tabulated the results.

In another experiment the inorganic phosphate determination was made as usual upon the acid ammonium sulphate filtrate immediately after preparation, and the amount found was 12.4 mg. The filtrate was allowed to stand at room temperature for 17 days and a second determination gave 15.6 mg.

The greater hydrogen-ion concentration of the trichloracetic acid filtrate may account for the difference in the increase of the inorganic phosphorus fraction.

In Table IV are recorded the results of phosphorus determinations made both upon the whole blood and the plasma of three patients. Trichloracetic acid was used as the protein precipitant.

REAGENTS AND SOLUTIONS

1. Ether-Alcohol Mixture—1 part of ether and 3 parts of 95 per cent alcohol, both redistilled.

2. Acid-Ammonium Sulphate—Saturated ammonium sulphate containing 15 c.c. of glacial acetic acid per liter. The C.P. ammonium sulphate was recrystallized twice from water and found to be free from all but traces of phosphates.

3. Trichloracetic Acid—This is a 20 per cent solution of trichloracetic acid.

4. Digestion Mixture—Equal parts of pure concentrated sulphuric and nitric acids.

5. Sugar Solution—1 per cent sucrose solution. To be made up fresh once a week.

6. Sodium Hydroxide—Approximately a 40 per cent sodium hydroxide solution prepared from sodium according to Bloor.

7. Phenolphthalein—0.5 per cent of pure phenolphthalein.

8. Dilute Sulphuric Acid—1 part of pure concentrated sulphuric acid and 3 parts of water.

9. Approximately an N/1 sulphuric acid.

10. Standard Phosphate—Stock standard contains 0.8340 grams of pure KH_2PO_4 per liter. The standard for use is made by diluting 25 c.c. of the stock solution to 500 c.c. One c.c. of the dilute standard is equivalent to 0.03 mg. of H_3PO_4.

11. Sodium Sulphite—20 per cent solution of sodium sulphite. This solution was made fresh at least twice a week.

12. Hydroquinone Solution—10.0 grams of hydroquinone per liter made slightly acid by the addition of 0.2 c.c. of pure concentrated sulphuric acid.

13. Ammonium Molybdate Solution—To 50 grams of pure ammonium molybdate dissolved in 300 c.c. of water are added 200 c.c. of water containing 75 c.c. of concentrated H_2SO_4.

All the water used in this work was collected and stored in glass water bottles.

When not in use the molybdate, phosphate, and hydroquinone solutions were stored in a refrigerator. The dilute phosphate solution was made fresh at least once a month.

SUMMARY

1. During the reduction of phosphomolybdic acid with hydroquinone an atmosphere of sulphur dioxide must be mantained to prevent oxidation by the air.

2. The sum of the lipoid and acid soluble phosphoric acid whether determined in the filtrate from the trichloracetic acid or ammonium sulphate precipitations, is practically equal to the total. The slight difference is attributed to experimental error. It might represent a fraction as nucleoportion which would be precipitated by either the trichloracetic acid or acid ammonium sulphate, and would also be insoluble in the ether-alcohol extract.

3. A procedure is described whereby a combination of the Bloor method with the Briggs modification of the Bell and Doisy method has been used for the estimation of total, lipoid and acid soluble, phosphoric acid in blood.

ADDENDUM

About the time this paper was sent to press one along similar lines appeared by Briggs.[3] In view of the fact that the somewhat different technic described above had already been used for seven months on a study on blood phosphates in tuberculosis, it was thought advisable to offer the same for publication.

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[2]Bloor, W. R.: Jour. Biol. Chem., 1918, xxxvi, 33.
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A SIMPLE DEVICE FOR RELOCATING MICROCOLONIES*

By F. T. Breidigam and T. M. Chang, Battle Creek, Mich.

IN working with microcolonies such as those of B. acidophilus in a mixed plate, one often experiences the difficulty of relocating the particular colony or colonies under study when such a relocation is desired. Marking with a wax pencil has been our usual practice, but this method gives only the approximate whereabouts of the colony, and not the exact location. To find the colony again in the marked circle usually takes some time.

With a simple device such as that illustrated in the accompanying figure, any microcolony in a plate can be given its appropriate "street number," and be relocated whenever desired. It consists of a semicircular celluloid

*From the Bacteriologic Laboratory of Battle Creek Sanitarium, Battle Creek, Michigan. Received for publication, June 26, 1924.

disk, cemented or attached by means of a set of screws to a semicircular metallic frame. The disk is made of an ordinary process photographic film. One quadrant of the disk is ruled into small squares, each square equaling one sq. mm. In each square there is one figure in the left lower corner, and another one in the right upper corner, and by a systematic arrangement of figures, such as that illustrated in the sketch, each square will bear a different combination of two figures, and by this combination any microcolony can be quickly relocated. The other quadrant is ruled so that it can be used as an ordinary bacterial counting plate.

When a colony is found which is to be relocated for further study, slip the disk over that half of the plate where the colony lies and in such a position so that the checkered quadrant will lie over the colony in question.

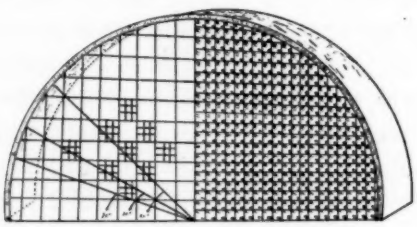

Fig. 1.

Be sure that the metallic frame is in close contact with the edge of the plate. With a wax pencil make a line on the plate along the diameter of the disk. Read the combination of figures in the square in which the colony is located, and this combination is the "address" of the colony. In reading the combinations of figures, it is best to read the figure in the lower left corner first, followed by the figure in the upper right corner. To relocate the colony put the disk over plate so that the marked line may lie along the diameter of the disk. Move plate around with the disk on top until the combination is found. Focus, and the colony will be seen.

This celluloid disk works excellently as a relocater of microcolonies, and serves also well as a convenient bacterial counting plate.

THE EFFECT OF HEAT ON STAINING PROPERTIES OF THE TUBERCLE BACILLUS*

By Thomas G. Hull, Ph.D., Kirby Henkes and Luella Fry, Springfield, Ill.

IT has become a common practice in many laboratories to subject to steam sterilizations specimens of sputum intended for examination for tubercle bacilli. The advantages of such a procedure are twofold, in that the specimen is not only easier to handle after the albuminous material is coagulated, but it is absolutely safe. Sputa without disinfectant (or with phenol 5.0 per

*From the Illinois Department of Public Health.
Received for publication, May 17, 1924.

cent) may be coagulated by steam heat and preparations made from the coagulum on microslides. If a little alkali is added to the sputum, as twentieth normal sodium hydroxide or an alkaline disinfectant, as cresolis compositus, before the specimen is subjected to the autoclave, the albuminous material is entirely liquefied making it suitable for concentration tests.

Staining in these laboratories is performed after a modified method of Spengler.[1] The autoclaved sputums are smeared on microslides with a little Mayers egg albumen as a fixative, dried and heated gently. Carbolfuchsin is applied to steaming eight to ten minutes, the specimens decolorized in acid alcohol (3 per cent hydrochloric acid in 95 per cent alcohol) until faintly pink; then counterstained thirty seconds in a saturated alcoholic solution of picric acid. The red tubercle bacilli stand out very distinctly against the yellow background.

Several experiments were conducted to determine whether there was any difference in the results of examinations on raw and cooked specimens, and if not, how much heat could be applied to sputum before the tubercle bacilli lost their acid-fast properties.

1. *To Determine the Effect of Coagulation.*—Thirty-seven specimens of sputum were selected at random as they were submitted by various physicians. All specimens were in 5.0 per cent phenol. They were divided into two parts, the cheesy particles of one being smeared raw on microslides, while the other was heated at fifteen pounds pressure of steam for forty-five minutes, the smears then being made and stained as described above.

In every instance the two examinations checked, 9 specimens showing tubercle bacilli present and 28 being negative. Since these results are in accord with those of Jones[2] and other workers, no further comparisons were made.

2. *To Determine How Long Sputum May Be Cooked.*—A pooled specimen of sputum was heated in the autoclave on successive days whenever opportunity permitted, smears being made and examined after each heating. On the twelfth heating, with a total of 8.5 hours, the organisms began to hold the stain weakly; at the fourteenth heating, with a total of ten hours at an average of eight pounds steam pressure, the tubercle bacilli were no longer acid-fast.

3. *To Determine the Effect of Heat in the Presence of Acid and Alkali.*—A pooled specimen of positive sputa was divided into seven parts: Three received equal amounts of sterile water, two received equal amounts of twentieth normal NaOH and two equal amounts of twentieth normal HCL. One of each of the above groups was left at room temperature, one of each heated at successive intervals in the autoclave and the last one with the sterile water placed in the incubator at body temperature. After a period of eighteen days all of the specimens at room temperature and one specimen in the incubator showed acid-fast organisms present. Fourteen heatings over the same period were given the other specimens under conditions similar to those in Experiment 1. After the eighth period in the autoclave the specimen containing twentieth normal HCL no longer showed acid-fast organisms. The

other two specimens—neutral and alkaline—showed acid-fast organisms to the end of the experiment.

4. *To Determine the Effect of Dry Heat.*—From a pooled specimen of positive sputa (previously cooked 45 minutes in autoclave) smears on ten glass slides were made. These were air-dried and placed in the hot air oven at a temperature of 154° C. to 156° C. Every ten minutes a slide was removed for staining purposes. At the end of the period—one hour and forty minutes—the organisms which had been subjected to the dry temperature for the entire time showed no indication of losing their acid-fast property. This indicates that haste in drying a smear of sputum for staining purposes, with incidental heating, does not injure it for the acid-fast stain.

5. *To Check the Accuracy of Tests Made on Specimens Heated with an Alkali.*—It has been shown that an alkaline disinfectant such as cresolis compositus[3] is considerably more effective in disinfecting sputum than is phenol. For this reason it has been customary in these laboratories to use the former material. When sputum is subjected to heat in such a solution it liquefies almost completely. After two or three hours standing, smears made from the sediment show tubercle bacilli in considerable numbers. There is the possibility, however, that when the organisms are few in number in a given sputum they will not sediment sufficiently by natural gravitation to be found in a smear of material from the bottom of the vial.

To determine the accuracy of the above method, the results of examinations made as described were checked with concentration tests. Sputa in cresolis compositus were sterilized in the autoclave and allowed to stand for two or three hours. The supernatant fluid was poured off and saved while smears were made from the sediment. Since the specific gravity of the tubercle bacillus is 1010 to 1080[4] there is the possibility that a solution too strongly alkaline will not allow the organisms to fall to the bottom. To reduce the specific gravity of the mixture, an equal part of alcohol was added before centrifuging (Loeffler used an equal part of alcohol containing a little chloroform). After twenty minutes centrifuging at high speed, the supernatant liquid was poured off and smears made of the sediment in the routine manner.

Of 490 duplicate examinations, 474 checked as being either positive or negative in each (102 positive and 372 negative), while 16 were at variance. Nine tests were positive by the direct smear or gravity method and negative in the alcohol centrifuge method, while seven were negative by the first method and positive by second. It would seem, therefore, that when sputum is dissolved in cresolis compositus 3 per cent solution, tubercle bacilli will sediment in the course of two or three hours by natural gravity, making the results of the tests as accurate as when the centrifuge is used.

CONCLUSIONS

1. Specimens of sputum subjected to steam sterilization are much easier and safer to handle and give as reliable results as do raw sputa.

2. Sputum may be sterilized as long as ten hours at an average of eight pounds steam pressure before the organisms lose their acid-fast properties.

3. In the presence of twentieth normal HCL six hours of sterilization sufficed to render the organisms no longer acid-fast. Twentieth normal NaOH apparently did not affect the acid-fast properties of the organisms either at room temperature or in the autoclave after repeated heatings.

4. Dry heat for one hour and forty minutes at 154° C. to 156° C. did not affect the acid-fast properties of the organisms.

5. Concentration tests may be made by liquefying sputum in the autoclave with cresolis compositus and allowing the tubercle bacilli to sediment by gravity.

Credit is due Mr. Alfred S. Harkness for his assistance in this work.

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[2]Jones: Jour. Lab. and Clin. Med., 1921, vi, 41.
[3]Park and Williams: Pathogenic Micro-organisms, edition 6, p. 392.
[4]Nebel: Arch. f. Hyg., 1903, xlvii, 57.

A SIMPLE AND RAPID METHOD FOR THE PREPARATION OF POLYCHROME METHYLENE BLUE AND THIAZIN RED. A RAPID METHOD FOR STAINING FROZEN SECTIONS WITH THIAZIN RED*

By Frederick Proescher, M.D., and Albert Paul Krueger, A.B.,
San Jose, California

THE polychrome methylene blue solution introduced by Unna for the staining of mast cell granules has found a wide range of usefulness in histologic technic. Originally empirically prepared by Unna[1] without knowledge of its chemical composition, many attempts were made by biologists and chemists to isolate and identify the specific component which gave to the solution its characteristic staining properties.

Romanowsky's[2] observation on the staining of the malarial parasite's chromatin substance gave the first impetus for inquiry into the composition of various commercial methylene blues as well as into that of polychrome methylene blue. This investigator found that eosin-methylene blue would sometimes stain certain cellular constituents of the Plasmodium malariae (later recognized as the chromatin substance) a distinct red color. He further determined that with a pure methylene blue the granules could not be stained. The particular brand of commercial methylene blue used together with the age of the solution were evidently the factors involved in production of the specific staining effect. The inconstancy of results obtained remained unsolved by Romanowsky and his method was almost forgotten.

Later on Ziemann,[3] in a painstaking investigation, tried to overcome the

*Received for publication, April 18, 1924.

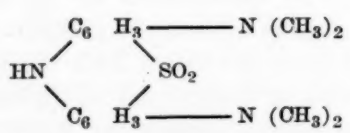
difficulties of the original Romanowsky method and found that by using methylene blue medicinale (Höchst) with eosin in certain definite proportions, a constant and distinct chromatin stain could be effected. He proffered the opinion that the specific dye responsible for the chromatin staining, and formed by mixing eosin and methylene blue together, was the so-called eosinic-acid-methylene blue.

At the same time, Nocht[4] started his investigation on the Romanowsky method and came to the conclusion that not the eosinic-acid-methylene blue but certain contaminations of commercial methylene blue in combination with eosin were essential for staining of the chromatin granules. He concluded also that Unna's polychrome methylene blue contained these decomposition products of methylene blue in large quantities. By using a neutralized solution of Unna's polychrome methylene blue (to eliminate effects due to the high alkali content) and mixing this with eosin, he obtained constant and distinct staining of the chromatin.

Ziemann[5] simultaneously reported that the addition of sodium borate (2-4 per cent) to methylene blue (Höchst) plus eosin gave constant results. Thus both methods were independent of the commercial brand of methylene blue and eosin used.

The specific dye concerned still remained unknown. Nocht's[6] further investigations demonstrated that a red derivative was formed by prolonged contact of methylene blue with alkali at room temperature, the action proceeding more rapidly at moderate heat. This substance, responsible for the specific staining of the protozoan nucleus, he designated for the time being as "Rot aus Methyleneblau." He gave as a mode of detecting its presence the following test: Extraction with $CHCl_3$ in which the dye is soluble with the production of a fiery red color throughout the extracting medium. Reuter[7] disputed that Nocht's "Rot aus Methyleneblau" was the specific factor in chromatin staining and contended that eosinic-acid-methylene blue alone was capable of producing the same staining effect. But his contention was erroneous and was disproved by Michaelis and Ziemann.

Finally Michaelis[8] pointed out that Nocht's "Rot aus Methyleneblau" was likely the so-called Methylenazur first prepared by the chemist Bernthsen.[9] Bernthsen had advanced the opinion that Methylenazur was simply an oxygen addition product of methylene blue (a methylene blue sulphone), and had attributed to it the following formula:

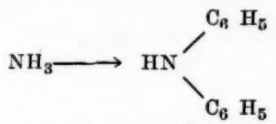


However, Bernthsen's formula was disproved by Kehrmann[10] who presented conclusive evidence that the oxidation did not lead to an addition of oxygen at the bridge of the methylene blue, but rather was due to a demethylation of one or two methyl radicals of the side chains.

CHEMISTRY OF THE METHYLENE BLUE AND ITS OXIDATION PRODUCTS

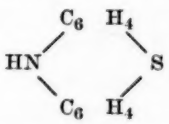
The following discussion is perhaps in order as furnishing a basis for the proper understanding of synthesis and oxidation products.

(a) *Synthesis of Methylene Blue.*—Starting with ammonia, two hydrogen atoms are replaced by phenyl groups:



Diphenylamine

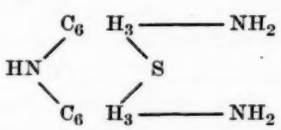
This chain becomes a closed ring by introduction of sulphur:



Thiodiphenylamine

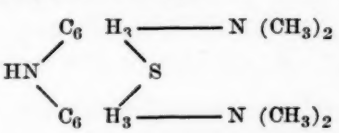
Thiodiphenylamine is known as Thiazin and forms the nucleus (chromophore) of all the methylene blue series of dyes.

The addition of two amino groups (auxochromes) in the side chains produces the simplest dye:



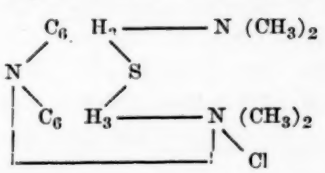
Leuco Base of Thionin

Substitution of the hydrogen atoms of the amino groups in the side chains by methyl radicals results in the formation of the so-called leuco methylene blue (or methylene white):



Leuco Methylene Blue

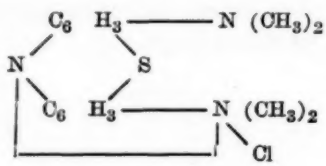
Treatment of this compound with an acid, e.g. HCl, gives methylene blue:



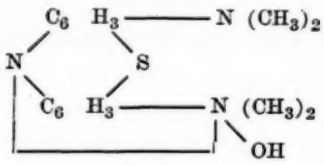
Methylene Blue

(b) *Oxidation Products of Methylene Blue.*—Oxidation of methylene blue results in the partial demethylation of the side chains, these latter being replaced by hydrogen atoms. The chief products of this demethylation process are trimethylthionin and asymmetrical dimethylthionin. The ratio of the dyes formed depends on the mode of preparation, the nature of the oxidizing agent being decidedly influential. The methyl groups split off are very likely oxidized to methyl alcohol and formaldehyde. It is possible that the demethylation does not stop with formation of dimethylthionin, but may proceed to monomethylthionin or thionin. These substances have

not yet been identified among the reaction products. The isolation of dimethylthionin by Bernthsen points to a complete destruction of the methylene blue molecule.

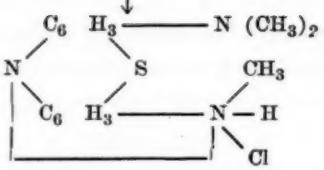


Chlorhydrate of Methylene Blue
(Blue)

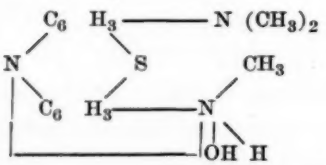


Free Base of Methylene Blue
(Blue)

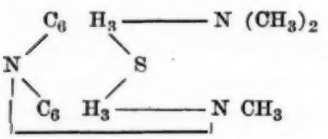
Demethylation derivatives
(Methylenazures)
Trimethylthionin)



Chlorhydrate of Trimethylthionin
(Blue)
Water-Soluble

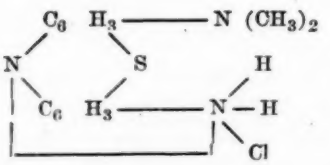


Free Base Hydrate of Trimethylthionin
(Blue)
Water-Soluble

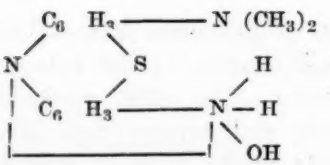


Free Base Anhydride of Trimethylthionin
(Red)
$CHCl_3$-Soluble

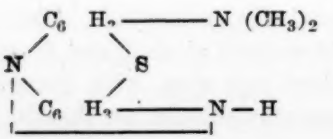
Dimethlythionin
(Asymmetrical)



Chlorhydrate
(Blue)
Water-Soluble



Free Base. Hydrate
(Blue)
Water-Soluble



Free Base. Hydrate
(Red)
$(C_2H_5)_2O$-Soluble

The ammoniacal character of methylene blue is changed by the oxidation process, and the resulting trimethylthionin and dimethylthionin derivatives have the properties of amines. While the free base of methylene blue is not precipitated by alkalies, and no color change occurs, the bases of tri- and dimethylthionin are precipitated as red colored anhydrides. These are insoluble in concentrated alkalies, but dissolve in chloroform or ether, imparting to the solution a cherry red color. Extraction of the ether or chloroform solution with water produces the blue hydrates which are soluble in the water.

Unna[11] proposed to include under the name, "Thiazin Red," the free bases of trimethylthionin and dimethylthionin. It may be more correct, however, to term these dyes "Trimethyl and dimethyl thiazin red." Commercial methylenazur is a mixture of trimethyl and asymmetric dimethylthionin, the latter predominating. Polychrome methylene blue solution contains besides both these substances unchanged methylene blue, some leucomethylene blue, and the decomposition products of methylene blue.

Polychrome Methylene Blue solution, e.g., thiazin red, has the peculiar property of staining certain cellular elements metachromatically; metachromatic referring to the staining of various cellular structures with different colors by a single chemical substance. An aqueous solution of polychrome methylene blue stains amyloid substance, mast cell granules, mucus and chondrium red, instead of blue. The above mentioned cellular elements must take up the anhydrides of the thiazin reds. The same phenomenon is observed if a pure watery solution of trimethylthionin and dimethylthionin is used.

It is a question whether the metachromatic properties of the thiazin bases can be explained as caused by differences in the color of their hydrates and anhydrides, or if there exists a tautomerie, e.g., intramolecular rearrangement of the hydrates and anhydrides due to different solvents.

PREPARATION OF POLYCHROME METHYLENE BLUE SOLUTION AND THIAZIN RED FROM METHYLENE BLUE BY OXIDATION WITH SODIUM PEROXIDE

For the preparation of polychrome methylene blue as well as thiazin red we found that Na_2O_2 could be used to considerable advantage in place of strong and weak alkalies, $K_2Cr_2O_7/H_2SO_4$, etc. The oxidation is very rapid and is in every way superior to that produced by the above mentioned agents. It may be carried on in an aqueous solution or may be performed in ethyl alcohol, acetone or chloroform.

Any good commercial brand of zinc chloride-free methylene blue (preferably "medicinale") may be used. In the event that a very pure polychrome methylene blue solution is desired, the methylene blue is best subjected to several preliminary recrystallizations from alcohol.

As stated above, the polychrome methylene blue solution, no matter what oxidizing agent is used in its preparation, contains varying amounts of methylene blue, leucomethylene blue, tri- and dimethylthionin (thiazin reds) and other decomposition products of methylene blue, in an alkaline solution of varying strength. The thiazin reds are most important for staining effects.

By oxidizing 100 c.c. of a 1 per cent methylene blue solution with different amounts of Na_2O_2 (5-100 mg.) the percentage of thiazin reds can be accurately controlled. Increasing amounts of Na_2O_2 cause the solution to change from a weak bluish red to a deep cherry red color.

A polychrome methylene blue solution suitable for pathologic work is obtained by using 20-30 mg. Na_2O_2 to 100 c.c. 1 per cent methylene blue solution. The Na_2O_2 is quickly weighed out to preclude any extensive reaction with the water vapor of the atmosphere and is added to the methylene blue solution. The mixture is immediately placed in a water-bath at 100° C. for fifteen minutes or may be boiled over a Bunsen flame for five minutes.

If a neutral solution of polychrome methylene blue or one of a particular alkalinity is desired, to meet the needs of special staining methods, the free alkali formed during the oxidation process may be neutralized with exact amounts of N/10 HCl. The necessary amount of acid is calculated from the following: 5 mg. Na_2O_2 : 1.18 c.c. $\frac{N}{10}$ HCl. HCl is for this procedure decidedly superior to organic acids, e.g., HCOOH, CH_3COOH, etc.

Where a stable polychrome methylene blue is to be made up, the alkali should be accurately neutralized since in alkaline solution, under the influence of atmospheric oxygen, unoxidized methylene blue is converted to thiazin reds which are insoluble in the presence of NaOH and precipitate, rendering the stain unfit for use. It is well to keep the neutral polychrome methylene blue solution in a paraffined bottle, otherwise alkali may dissolve out of the glass and produce oxidation.

PREPARATION OF THE THIAZIN REDS (METHYLENAZUR)

Since the specific staining properties of polychrome methylene blue are dependent upon the thiazin reds, it is obviously preferable to employ a solution containing these dyes only in place of a mixture of these substances in varying amounts with undesirable decomposition products of methylene blue.

Following is a simple and rapid method for the preparation of thiazin reds:

Fifty grams of methylene blue are dissolved in 200 c.c. water (using a 500 c.c. beaker) and the solution heated to 75-80° C. Five grams of Na_2O_2 are dissolved in 20-30 c.c. water and quickly added to the warmed methylene blue. The reaction takes place immediately with resultant precipitation of the free thiazin bases as large bluish-red masses exhibiting a peculiar metallic luster. The solution when cooled is filtered through a Buchner funnel and the precipitate dried at 37° C. After 24 hours at this temperature it is advisable to break up the large lumps in a mortar and to dry again. The fine powder obtained is completely dried over $CaCl_2$ or concentrated H_2SO_4 and kept in a tightly stoppered bottle.

A suitable staining solution is prepared by dissolving 0.5 gm. of the dried bases in 100 c.c. distilled water. The weighed quantity of dye is ground in a mortar with small amounts of water until completely taken up. The solution is then diluted to 100 c.c. and filtered. A deep azure-blue solution is obtained which keeps perfectly in a paraffined bottle.

STAINING OF FROZEN SECTIONS WITH THIAZIN RED

The above described aqueous solution of thiazin red is well adapted to the staining of frozen sections. However, when used for this purpose, it is best to employ 1 per cent acetic acid instead of distilled water as a solvent. Five-tenths gram thiazin red is dissolved in 100 c.c. 1 per cent acetic acid (made by diluting 1 c.c. glacial acetic acid to 100 c.c.).

This slightly acid solution gives very sharp differentiation of the nuclei of epithelial cells and leucocytes. Connective tissue remains either colorless or is stained very slightly blue or metachromatic violet. Sections remain ten to twenty seconds in the staining solution, are washed with water and mounted in glycerine or better levulose syrup. Immersion of the section for even five to ten minutes in the staining solution will not result in overstaining, for a thorough washing in water gives an excellently differentiated picture. Sections may be kept some time if mounted in thick levulose syrup and framed with asphalt lack or paraffin.

The procedure described here is especially suited to formalin fixed material. Frozen sections cut from tissue which has not been previously treated with formalin, should be immersed in 5 per cent formalin solution for ten to twenty seconds and placed directly in the staining mixture without washing in water.

SUMMARY

1. The historical development of polychrome methylene blue together with the general chemistry of methylene blue and its oxidation products is outlined.

2. A rapid and convenient method for the preparation of polychrome methylene blue solution and thiazin reds from methylene blue by oxidation with sodium peroxide is given.

3. A discussion of metachromasie and the application of a solution of thiazin reds to frozen section technic conclude the paper.

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A TETANUS TOXIN FILTERING APPARATUS*

By Edward Redowitz, Glenolden, Pa.

IN the routine production of tetanus toxin, the process of filtration is one of the most painstaking and needlessly difficult tasks. The handling of living tetanus cultures with the toxin, and the forcement of the sticky fluid through filter candles present the most objectionable features of the operation. Some laboratories seek to obviate the latter difficulty by several preliminary filtrations. This requires more manipulation of the culture than would be desirable and at the same time may reduce the potency of the toxin. The apparatus described in this paper was designed with all these points in mind, and for the past three years has been used with marked success.

The machine is simple in construction and easy to operate. The culture must be poured only once, and within two or three hours, using a triple machine, thirty or forty liters of toxin can be filtered without the danger and delay of other methods. The main feature of the apparatus is the use of percolators or large earthenware funnels as sheaths for the candles. The candles are encased in the lower, narrow part. Above the candles in the upper portion, filters consisting of layers of paper pulp serve to remove most of the organisms and slimy substance before the filter candles are reached.

The apparatus may be set up on any ordinary wooden laboratory table, as illustrated. Holes are made in the table to fit the lower ends of the percolators. A wooden stand, six inches high, with larger holes, is attached to the table. This will hold the percolators firmly in position. All the parts are durable. The preparation of the paper pulp requires a little time, but it can be made in large quantities and kept ready for use in a suitable container with a tight lid.

The number of percolators to be used will be governed by the quantity of toxin to be filtered. One percolator set up complete will hold about four liters of fluid, and, by means of water vacuum, enough toxin can be drawn through to fill two nine liter bottles. The pulp and the filter candle will then become clogged and should no longer be used.

After using the apparatus the percolators with their contents can be placed in a can and autoclaved. The rubber stoppers holding the candles should be removed from the percolators, and to save them from melting, must be autoclaved in a vessel containing water. No other connections need autoclaving.

The parts necessary for one percolator are:

1. A cone shaped stoneware percolator.
2. A Berkfeldt, or a Mandler filter candle 2 × 10 inches.

*From the Mulford Biological Laboratories, Glenolden, Pa.
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3. A one-hole rubber stopper size No. 10 to hold filter candle in percolator.

4. Galvanized wire screen with a rim, for bottom of percolator.

5. Filter paper cut round to fit on wire screen.[4]

6. Paper pulp soaked in 0.5 per cent solution of phenol.

7. Filter paper cut round to fit on top of paper pulp.

8. Round galvanized wire screen to be used as weight on top of last layer of filter paper.

9. Two pieces of rubber pressure tubing connected to glass tube elbows that fit in a rubber stopper.

10. A sterile bottle.

PREPARATION OF PULP

Ordinary filter paper is torn in long strips, two or three inches wide, placed in an agateware can. It is then covered with 0.5 per cent solution of phenol, and allowed to stand overnight. The next morning the phenol solution is poured off completely. The wet paper is then ground in a mechanical meat grinder, or if a meat grinder is not available, the paper can be rubbed into pulp on an improvised grater made, for example, of a large galvanized can lid with holes punched so that the sharp edges stick up. The wet pulp is then partially dried in an incubator.

Fig. 1.

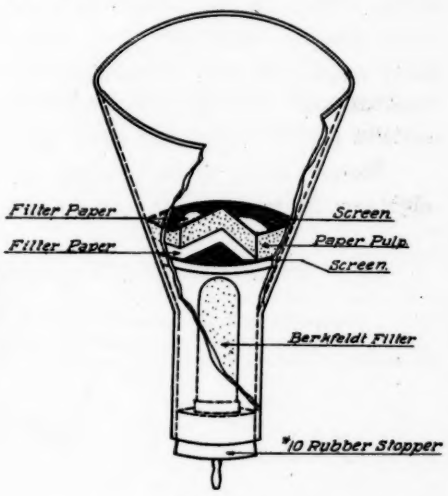

Fig. 2.

The proper degree of dryness is important, for if the pulp is too wet, it will pack down so tight in the percolator that it will interfere with the free flow of the fluid. On the other hand, if the pulp is too dry, it will not be an effective filter.

The pulp is spread out on paper in an incubator and allowed to remain for one or more days, until it becomes so nearly dry that an ordinary pressure of the hands will not squeeze fluid out of it, but it must feel moist to the hand. When pressed and moulded, it should hold together in a lump.

If it crumbles, it is too dry. The finished pulp is placed in an agateware can with a tight lid, and kept in a cool place.

SETTING UP THE APPARATUS

After the pulp has been prepared and all the necessary parts assembled, the apparatus may be set up when needed. The only parts necessary to be sterile: filter candle (*2*), rubber stopper (*3*), rubber tubing (*9*), and receiving bottle (*10*). The filter candle may be wrapped in paper and autoclaved, and all rubber stoppers and rubber tubing can be sterilized by boiling in a suitable vessel just before use.

The stem of the sterile filter candle (*2*) is passed through the sterile rubber stopper (*3*),which is inserted securely into the narrow end of the percolator. The percolator is then placed in position. The paper filter is now arranged above the filter candle, the parts being added in the following order: (*4*) The wire screen. (*5*) Two layers of filter paper. (*6*) Three or four inches of paper pulp, packed tightly by means of a small heavy glass bottle with a round bottom or some other flat bottomed instrument. (*7*) Another double layer of filter paper and finally the wire screen. (*8*) The filter candle is now connected by means of sterile rubber tubing with the vacuum tube and the sterile bottle. The percolator is filled by pouring the culture slowly along the side, the vacuum pump is started.

Note: With from twenty to twenty-eight inches of vacuum, nine to eighteen liters of toxin can be drawn through each filter.

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EDITORIALS

The Reaction to Syphilitic Infection

THE evolution of methods for the isolation and growth in pure culture of the Spirochete pallida inaugurated an intensive study of experimental syphilis in the rabbit, the results of which are now beginning to assume proportions of some significance.

Predominant workers, of whom there are many in this field, are Brown and Pearce, whose contributions to the subject have opened up avenues of investigation which have a direct and definite application to the clinical recognition and handling of this infection.

In spite of the antiquity of syphilis and the intensive and extensive study to which its manifestations have been subjected, there are still many phenomena associated with this disease the mechanism of which remains to be elucidated; for example, the factors influencing the pleomorphism of its clinical manifestations, or those determining the incidence of neurosyphilitic involve-

ment; what determines the latent periods of the infection—which are undoubtedly more frequent than has hitherto been supposed—and the like, answers to some of which problems are beginning to appear possible.

A factor of importance as related to the widespread incidence of syphilis and a possible explanation of those cases in which meticulous examination has failed to demonstrate the primary lesion, is found in the observation that highly virulent strains of Spirochete pallida may and can penetrate the normal, unbroken mucous membrane of the rabbit with resultant generalized syphilis but without the prior production of a gross lesion at the portal of entry.[1]

The clinical failure to prevent the disease in olden days by excision of the chancre and the failure of prophylactic measures when unduly delayed are explained by the experimental demonstration of the rapidity with which the spirochetes migrate and are disseminated from the site of invasion, as it has been shown that spirochetes are constantly present in the regional lymph nodes within forty-eight hours after inoculation and long before there is any evidence in the slightest degree of abnormality at the site of invasion.[2]

It has also been demonstrated that the pathway by which this dissemination takes place is primarily the lymphoid structure, though spirochetes have also been isolated from the blood in the rabbit, seven, ten, and thirty days after intratesticular inoculation, periods corresponding to twenty-six, twenty-three, and three days prior to the appearance of the initial lesion. Invasion of the blood and lymphoid system, therefore, occurs early in the incubation period of the disease.[3]

The relation of the lymphoid tissues to spirochetal infection is very interesting and of great practical importance. The adenopathy of syphilis has long been familiar but its importance and exact relation to the disease has, perhaps, not been as well appreciated and certainly not previously as clearly demonstrated. The importance of the lymph structures as avenues for the early dissemination of the spirochetes in syphilis has been shown by Eberson,[3] and by Brown and Pearce,[2] who emphasize the significance and importance of these structures as "reservoirs" of syphilitic infection. The constancy of this early localization of the spirochetes in the lymph nodes, their presence as early as five days after inoculation, and the fact that such localization serves as an effective focus for the maintenance and systemic dissemination of the disease, constitute important observations of definite practical application. Of even greater importance, however, is the demonstration that lymph node infection may survive and persist for long periods during which the disease is latent and entirely asymptomatic, which has been indisputably shown in the rabbit.[2]

That similar conditions obtain in the human being, has been shown by the studies of Engman and Eberson[4] concerned with the infectivity—as determined by rabbit inoculation—of the blood, semen, nasal washings, and lymph nodes of a series of cases latent from one to forty years, these being selected from a series of five hundred syphilitics, 15 per cent of whom were latent and asymptomatic.

In five of fourteen cases viable and virulent spirochetes were found on

the examination of a single lymph node, and in two out of seventeen cases spirochetes were found in the semen. How many more positive findings might have resulted from repeated examinations it is impossible to hazard. The demonstration of the spirochetes in the semen is an observation of decided interest.

Comparable findings are reported in the rabbit, in which, "though spontaneously clinically recovered," and showing no clinical or pathologic evidences of infection other than an enlargement of the popliteal nodes, viable and virulent spirochetes were found in these structures from which observation it was suggested that the possible seats of predilection for spirochetes during the latent periods and the chief sources or reservoirs of infection were the lymphoid structures, either massed as nodes or as simpler perivascular lymphatics.[5]

Experimental evidence is also available to indicate that the invasion of the central nervous system may take place through the lymphatic system[6] and, as is now a matter of clinical knowledge, that such invasion may take place early in the disease.

Recalling the fact that, while there is no unanimity in regard to satisfactory criteria for the cure of syphilis, a frequently adopted standard has been the success or failure of reinoculation, Chesney and Kemp[7] suggest the possibility of the utilization of the lymphoid tissues as test materials in determining the efficiency of therapeusis.

The problem attacked by these investigators was to determine if it were possible through the use of arsphenamine to render syphilitic rabbits free from spirochetes as determined by lymph node transfer, and whether such "cured" rabbits were susceptible to a second inoculation with the same strain.

Their conclusions follow:

"1. Six doses of arsphenamine (10 milligrams per kilo) 127 days after infection are sufficient to render the popliteal lymph nodes incapable of transmitting the infection to normal animals.

"2. Such rabbits, as judged by the absence of local lesions, are refractory to inoculation.

"3. Untreated rabbits were also refractory to inoculation.

"4. The refractory state is probably due, either to an acquired immunity, or to the persistence of living foci elsewhere in the body."

Reinfection or superinfection, in the light of the experimental evidence available, can no longer be looked upon as indisputable evidence of the prior total eradication of syphilis, for experimental demonstration of superinfection in the rabbit has been achieved and explained on the assumption that the resistance acquired as a result of infection with an organism of low virulence, may never reach the point of effectual protection against an organism of high virulence.[8]

Experimental superinfection is also reported by Eberson,[9] who concludes that a state of resistance does not necessarily prevent reinoculation but appears to favor a localization of the organism in the tissues without manifest lesions. Isolated clinical reports of probable reinfection can be found in the current literature, the most clear-cut case recently available being that re-

ported by Shinkle[10] in which a typical acquired chancre containing Spirochete pallida was demonstrated in a negro aged sixteen with a history of congenital syphilis corroborated by the presence of clinical and laboratory evidence of general paralysis.

The mechanism of reinoculation or superinfection is closely related to immunity in syphilis which has not been neglected in experimental studies. Whether or not a second (superinoculation) inoculation in the rabbit is capable of further participation in the disease or, because of acquired resistance through the first infection, limited to the production of local reactions, has been studied by Brown and Pearce.[11]

Their conclusion was that the ultimate lesions are probably due to the combined action of both strains.

According to Eberson,[12] the elaboration of syphilitic antibodies appears to be at a maximum, late in the disease, predisposing to a condition of latency without demonstrable lesions, though spirochetes could be found, the lesions following inoculation with such "latent" strains showing a definite tendency toward a chronic proliferating character.

A possible explanation of this tendency of syphilitic lesions toward involution *in situ*, as advanced by Eberson and Engman[13] lies in the probable formation of local antibodies which slowly destroy or tend to repress the activity of the spirochetes.

Chesney has studied the influence of age, sex, and method of inoculation on the course of syphilitic infection in the rabbit and concludes that intradermic inoculation is less prone to cause generalized syphilis in the rabbit than intratesticular inoculation; that the primary inoculation in females gives rise to a less marked reaction than in males; that young rabbits were more prone to marked initial lesions with slight tendency toward the postponement of general lesions which were less severe in character and extent than in older animals. He also found that the suppression by castration of the initial lesion did not lead to the increased incidence or early appearance of subsequent generalized lesions during an observation period of ninety days and that intradermal inoculation with little or no local reaction might give rise to general invasion as evidenced by positive lymph node transfer even though no local lesions might be observed.[14]

Studies of animal resistance to syphilis have opened up interesting bypaths among which may be noted the possible relationship and influence of the endocrine glands to the reaction to syphilis.

It is well known that the endocrine balance is subject to variations during pregnancy and studies in the human being have shown an association between the phenomena concomitant with pregnancy and the reaction to syphilitic infection which are borne out by the results of observations in experimental syphilis in the rabbit in which it has been found that the reaction to genital infection coincident with conception varies from the reaction in the normal animal similarly inoculated, and that this difference, moreover, extends well into the period of lactation.[15]

A similar study by Moore[16] in the human being may be thus summarized:

If impregnation and infection practically coincide, or if infection occurs during pregnancy, the patient may develop the usual manifestations of syphilis which, however, are much milder than if the infection is independent of pregnancy.

Some cases, when infection occurred about the time of impregnation, failed entirely to develop the usual early lesions of syphilis, and in a few cases (three out of two hundred), the response to infection acquired at the beginning of or during pregnancy was markedly altered, the usual time elapsing between the primary and secondary lesions being much prolonged, or the interval between the secondary and tertiary lesions being much shortened. The protection afforded by pregnancy in some cases was shown to be prolonged and the suggestion is advanced that the explanation might lie in some change in the endocrine system or in various changes in the balance of metabolism dependent upon or associated with the occurrence of pregnancy. These theories, however, as well as that of the possible influence of the chorionic ferments—the very existence of which has yet to be proved—remain to be demonstrated.

That some relation exists between endocrine efficiency and the phenomena of syphilis appears probable on experimental and inferential grounds, but just what this relation may be and what its modus operandi is, as yet, is unproved.

That the endocrine balance is, at times, disturbed in pregnancy is a matter of fact and there may be some relation between this imbalance and the variations in pregnancy of the reaction to syphilitic infection. Moreover, experimental data show that the status of the endocrine glands varies with different stages of syphilitic infection, and that they appear to be on a higher plane of functional activity (thyroids, parathyroids, adrenals, and thymus); still further, the course of the disease can be modified by operative interference with the glandular system, or by the administration of chemical agents effecting changes in the endocrine glands and lymphoid tissues, findings which may have some bearing on the abnormalities present in congenital syphilis.[17]

As neoplasms are characterized by lawless and irregular growth, and as certain of the phenomena associated with normal growth are related to endocrine structures, the occurrence of a spontaneous neoplasm in the scar of an old scrotal syphilitic lesion in the rabbit is of interest.[18]

The protean character of the lesions of syphilis has more than once given rise to the suggestion that there were possibly varying strains of Spirochete pallida with different predilections. While definite evidence for or against this supposition is yet to be adduced, and the question is still an open one, it is beyond cavil that variations in the clinical manifestations of syphilis may also be influenced by other factors, such as the reacting mechanism of the host and the disease-producing properties of the infecting strain.

Experimental study of syphilis in the rabbit has shown beyond question a relation between the defensive reaction and mechanism and the degree

of local reaction at the site of invasion, and studies of the effects consequent upon a modification by various means of this local reaction have shown that it is, in this manner, possible to vary the types of general reaction produced and to protect certain groups of structures—bones, for example—by allowing the testicular initial lesion to progress to a given point.[19]

It is held possible, therefore, that a modification of the defensive reaction influences the clinical variations of the disease while also admitting the possibility that biologic variations in the infecting organisms may contribute still further to variations in the type of syphilis produced.

The idea of an inhibitory influence has more than once been suggested as an explanation of the various phenomena of syphilitic infection and such effects have been strikingly shown in experimental infections in the rabbit in which scrotal or testicular lesions giving extremely marked reactions at the site of inoculation have been accompanied by total absence of the general manifestations of the disease.

An investigation conducted to determine the influence of a local reaction in one part of the body to manifestations of the disease elsewhere by means of a reduction or suppression of the local reaction at the site of inoculation rather definitely demonstrates the relation between the intensity of the local reaction and the protective mechanism invoked by it, as a reduction of the local lesion by unilateral castration led to an increase of the generalized lesions and the total suppression of the primary lesion by therapeutic agents also led to an increase in the general manifestations of the disease.[20]

The conclusions drawn from the experimental work in this connection were that the reaction at the site of inoculation tends to dominate the entire course of the infection, either inhibiting or obviating the necessity for the development of lesions elsewhere; and, conversely, that reduction or suppression of this local reaction by any means which does not also exercise an equal effect on the spirochetes removes this control and tends to increase the activity and severity of the infection and the occurrence of general lesions. These observations, while not in entire accord with those of Chesney, previously noted, cover a larger series of experiments over a longer period of observation and the conclusions drawn seem warranted by the data presented.

These experiments and conclusions have a very definite relation to the clinical management of syphilis as indicating that partial or subcurative treatment, by repressing the local reaction without coincidently eradicating all foci of spirochetes, may directly contribute to more severe generalized types of the disease and there are reports of clinical experiences in conformity with this conception.

This has been experimentally demonstrated in rabbits which, under these circumstances, have even been shown to be more susceptible to reinfection than normal animals.[21]

These and other studies in the reaction to syphilitic infection, while far from entirely elucidating the mechanism concerned, are of definite value

and interest and warrant the hope that future work may lead to a clear understanding of the processes concerned with the consequent development of clinical methods of exactitude in treatment and control.

Not the least important of the deductions to be made from the work outlined is that variations in the degree and extent of infection in the experimental animals represent an orderly expression of the interaction between two sets of forces: "one directed toward the spirochete itself, the other whose object is the neutralization of toxic or harmful effects. These reactions proceed in parallel directions but not to an equal degree," for, while the defensive reaction may be carried to the point of complete neutralization of the power of the spirochetes to produce disease, infection is never thus entirely eradicated but merely remains latent.

"If one may regard a lesion as primarily an expression of toxic injury on the one hand and of a defensive or protective reaction on the other, an analysis of the phenomena of periodic development and resolution of individual lesions leads to the conclusion that there is a quantitative relation between the injury produced by the spirochetes and the reaction of the host; that when the injury has been checked, the reaction tends to decline; but as not all spirochetes are destroyed in this process, the cycle is repeated, the state of resistance mounting with each successive repetition until the surviving organisms are incapable of producing further injury."[22]

From these considerations have been developed the "law of inverse proportions" and the "law of progression or sequence."

The "law of inverse proportions" expresses the thought that the duration of any active manifestations of the disease, (lesion), is inversely proportional to the intensity and extent of the initial local reaction; and the expression of this law has been experimentally demonstrated by the experimental modification of the course and general characteristics of experimental syphilis almost at will by means of modifications in the degree and extent of the local reaction, subject to the second general principle expressed as the "law of progression."

The "law of progression" is based upon the fact that "when allowed to pursue an undisturbed course, syphilis tends to preserve an orderly progression with varying degrees of intensity and extent. This characteristic of the disease appears attributable to the fact that different groups of tissues are not equally adapted to the growth and multiplication of the spirochetes, on the one hand, and that they are not equally sensitive or reactive to the toxic effects of the organisms on the other. Moreover, there is a natural order of susceptibility and of involvement, and the protective influences arising from reactions taking place in one group of tissues is extended to others in an equally orderly fashion. Under normal circumstances, therefore, the sequence observed in the occurrence of manifestations of the disease may be regarded as an orderly progression whose direction is determined by the relative susceptibility of different tissue groups and whose limits are fixed by the sequence and extent of the defensive reactions."[22]

It is, of course, obvious that as the manner and method of attack are varied, so there will be corresponding variations in the degree and extent

of the defensive reactions, "hence, it may be said that the manifestations of the disease presented in any given instance depend, not only on the general laws which govern syphilitic reactions, but also on a number of other circumstances which includes any and all conditions affecting the initiation of the infection, the resistance of the host, and the pathogenic properties of the organism themselves."[22]

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—*R. A. K.*

The American Society of Clinical Pathologists

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Teamwork of the Surgeon and Pathologist

The American College of Surgeons recognizing the importance of the clinical laboratory on the scheme of hospital standardization and the influence of the American Society of Clinical Pathologists in elevating the scientific and social status of the clinical pathologist has adopted the custom of inviting a representative of the A. S. C. P., at its national and sectional meetings.

Our secretary, Dr. Ward Burdick, read a paper before the Rocky Mountain Section of the A. S. C. P., which met at Pocatello, Idaho, last summer and delivered an address on the subject of "Financing the Hospital Laboratory" at the Clinical Congress of the American College of Surgeons, recently held in New York City. The surgeon, no less than the internist, appreciates the services of the clinical pathologist in helping him arrive at a diagnosis. The hospital laboratory has become a meeting place for the clinicians where they hold consultation with the pathologist on their cases with greater benefit to the patients. It makes an ideal arrangement, removing the impersonal element in laboratory work and introducing the human factor, at the same time directing the talents of both the clinician and the pathologist to the formulation of a correct diagnosis.

Membership in the A. S. C. P.

The remarkable achievement of the society in raising the status of the clinical pathologist from his former humble station as a technical aid to the eminent position of consultant has received due recognition from the best clinical pathologists of the country who have enrolled under its banner.

It is not the aim of the society to constitute an aristocratic or exclusive organization, and it therefore extends an invitation to medical graduates who are devoting all, or the major portion of their practice to clinical pathology to become members and participate in its activities. Further details as to qualifications may be had by corresponding with the secretary, Dr. Ward Burdick, 652 Metropolitan Building, Denver, Colo.

Standardization of Laboratories

The evolution of the clinical pathologist as a specialist has not followed along the same lines as his older confreres in the fields of surgery, laryngology or ophthalmology. It was taken for granted that the surgeon, the laryngologist or the oculist must be first of all a graduate physician and then have acquired the science and skill of his respective specialty. Little attention was paid to his instruments and armamentarium, not so, however, with the clinical pathologist. From the very genesis of this branch of medicine as a separate specialty, emphasis has been laid on his equipment, that is on the laboratory,

rather than on the intellectual and scientific endowment of its director. We are unfortunately wont to speak of the "laboratory," probably as a figure of speech, when we actually mean the clinical pathologist. The result of this misapplication of words has produced the psychologic impression that the physical laboratory with its instruments and reagents is the important factor, while the one who carries out the determinations and interprets the findings is a secondary proposition.

It is not surprising, therefore, that early in the development of our specialty, abnormal manifestations began to appear. Instead of a healthy growth patterned after the evolution of the other fields of medicine, degenerative tendencies showed themselves, becoming plainly evident by a commercialization of this specialty. The outward signs were advertising through the mail and in medical journals and the formation of "chain of stores" laboratories. The general practitioner seeing these advertisements in respectable medical journals was unconsciously led to believe as it were, that the laboratory was a sort of contrivance where you placed a specimen in the hopper and received a diagnosis at the other end. The personal element was left out of account. Little wonder, then, that irresponsible laboratories, often conducted by incompetent lay technicians sprang up in the larger centers of population and by the usual methods of cut throat competition, fee splitting, contract practice and in general resorting to unethical measures, have produced a deplorable situation in some parts of the country resulting in great harm to patients and loss of prestige and usefulness of the ethical clinical pathologist.

The American Society of Clinical Pathologists, has from its very inception, led an active fight against the morbid manifestation in the development of this important field of medicine. Our members will recall the bulletins issued by our organization dealing with the advertising evil. As a result, the medical journals, while not altogether discontinuing laboratory advertisements, have entirely stopped the publication of fees. The cancer is, however, not all eradicated, and therefore, deserves the attention of all who are interested in scientific medicine. The American Medical Association has recognized the seriousness of the situation by appointing a special committee to look into this question. Evidence of the activity of this committee is shown in a questionnaire that is being sent to clinical pathologists throughout the country asking for data regarding their laboratories. While allowing full credit for the good intentions of the committee, the personnel of which has our highest respect, may we in all humility call attention to the fact that here too, the center of gravity seems to rest on the laboratory qua laboratory and its equipment. Data are requested for the name of the "Institution," the housing, space occupied, dimension of rooms, equipment, etc. Questions are asked whether the laboratory does this or that kind of work? At the same time it is only fair to mention that there is a query as to the personnel: Is the director a medical graduate, his education, experience, duties, fraction of time spent in laboratory.

We do not desire to be captious or pedantic when we insist that the laboratory *per se* is secondary to the clinical pathologist. It is not mere word splitting. The American College of Surgeons when they inquire into the qualifications of an aspiring applicant for fellowship make no inventory of his surgical instruments or, similarly the American College of Physicians do not look into the office equipment of their applicants. The same is true with the ophthalmologist and laryngologist. It is the man behind the gun. We should hold the clinical pathologist responsible for his work which, of course, necessitates an adequate equipment. A mere collection of glistening bottles, a gaudy array of microscopes, microtomes, and colorimeters on glittering glass tables does not necessarily betoken exact and reliable findings and interpretations. One would rather be operated upon on a kitchen table by a skillful surgeon than in the best equipped operating room by a novice.

It is not our intention to put any impediments in the way of the good work of the committee, but rather to urge our members to fill out the data requested as their compilation will serve a most useful purpose. The questionnaire has but been a peg on which we could not resist hanging our pet thesis: It is the physician at the head who makes the laboratory.